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DISSECTION OF THE ATRIO-VENTRICULAR NODE AND BUNDLE IN THE HUMAN HEART

By E. W. WALLS, *Department of Anatomy, University College, Cardiff*

Doubt has recently been expressed by certain workers whether the atrio-ventricular node and bundle are present in the human heart. Robb & Robb (1942) stated: '...but whether a similar system composed of either Purkinje, Purkinje-like, nerve or even ordinary cardiac muscle cells, exists in the human heart is still being questioned.' Glomset & Glomset (1940*b*), after dissecting more than forty human hearts, agreed with Mahain (1931) that it is impossible to make a gross dissection of the bundle in man, and they are quite definite that the structure frequently seen through the endocardium of the left ventricle, and described as the left limb of the atrio-ventricular bundle, is not the left limb at all but simply fasciculi formed by components of the septal musculature. Moreover, only ordinary cardiac muscle fibres could be found microscopically, although many blocks were prepared, including some from the upper part of the interventricular septum. Todd (1932) and Glomset & Glomset (1940*a*) challenged the existence of the sinu-atrial and atrio-ventricular nodes. Todd said: 'I can find no special nodal tissue and do not believe that either sinu-atrial or atrio-ventricular node exists as a special formation: any specificity of these regions must be purely physiological; it has no basis in anatomical fact.' Doubt has therefore been cast on the accounts in our text-books in which the human A.V. node and bundle, the limbs of the bundle and their terminal subendocardial distribution as a network are described and illustrated. However, Davies (1942) described S.A. and A.V. nodes, the bundle, its limbs and their subendocardial ramification in the human heart. He also stated that typical large Purkinje fibres are present in the human heart but that they only appear some way down the limbs, the proximal parts of the limbs being made up of ordinary ventricular muscle fibres.

The evidence in favour of the existence of the conducting system in the heart of man is plentiful, e.g. the results of careful dissection, the microscopic appearance in serial sections of entire human hearts, comparison of human electrocardiograms with those of animals in which there is no doubt concerning the existence of the system, comparison of abnormal human electrocardiograms with those of animals in which experimental lesions have been produced, and correlation of post-mortem findings such as calcification, thrombosis, infarct or tumour involving parts of the heart in which the conducting tissue is certainly present in other animals, with the clinical condition.

In many post-mortem examinations, however, no lesion can be demonstrated in the heart in agreement with the bundle lesion diagnosed clinically; nevertheless, to exclude such a lesion would necessitate complete serial sections of at least a very large block from the heart in question.

The majority of anatomists rely on comparative specimens for demonstration purposes and, indeed, few will admit to having seen the bundle in the cadaver. Another point of importance is the complete lack of convincing photographs, as distinct from diagrams, of dissected human specimens.

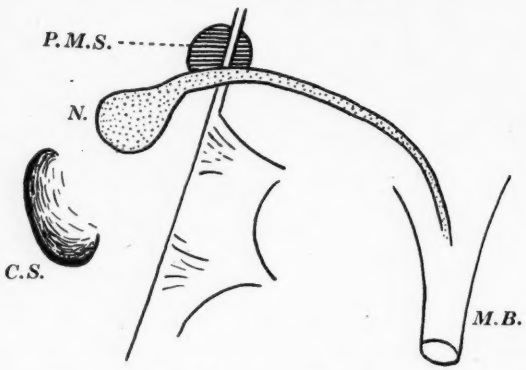
PRESENT WORK

Twenty hearts were obtained from the post-mortem room and dissected either fresh (14) or after fixation in 5 % formalin (6). Results were achieved more easily in fresh hearts than in fixed, in agreement with Curran (1909) but not with Keith (1906). The hearts had been opened by the pathologist, care having been taken to avoid damaging the interventricular septum. With the heart pinned out the right atrium and right ventricle were carefully examined for any naked eye evidence of conducting tissue. In a few cases part of the right limb showed as a thin white strand running into the moderator band, or, when that structure was absent, upon the muscular ridge continued from the supraventricular crest. In two specimens, hearts 16 and 18, the entire right limb could be followed easily without any dissection from in front of the pars membranacea septi to the anterior papillary muscle of the right ventricle. In heart 16 a delicate subendocardial network was seen on the free wall of the right ventricle. Pl. 1, fig. 1, is a photograph of heart 18 showing the entire length of the right limb of the bundle. In this specimen the moderator band was well marked, unusually so for a human heart.

In most hearts, however, nothing was obvious to the naked eye and the bundle had to be exposed. To do this the septal cusp of the tricuspid valve was removed and in the angle between the attachment of the cusp and the pars membranacea septi the A.V. bundle was found, either just under the endocardium or covered by a varying thickness of

the muscle of the upper part of the muscular septum (Text-fig. 1). The size of the membranous part of the septum varied greatly and when it was small the ventricular muscle extended higher, a fact which made it more difficult to find the bundle. This was also noted by Curran (1909) who, nevertheless, dissected the bundle successfully in every heart of a series of 96. Holmes (1921) and Keith (1906) both stated that in many cases it was quite impossible to demonstrate the system by dissection, and in the present series of twenty hearts two showed no trace of conducting tissue.

After exposure the bundle could be followed back to the right atrium through the trigonum fibrosum dextrum, a procedure which varied in difficulty according to whether the bundle lay deeply in the fibrous body or well over to its right side. In some



Text-fig. 1. Simplified view of the right A.V. junction to show the position of the A.V. bundle. *C.S.* coronary sinus. *M.B.* moderator band. *N.* A.V. node. *P.M.S.* pars membranacea septi.

hearts the fibrous body was very dense, approaching in consistency to that of cartilage, and it was then not easy to avoid damaging the bundle. Within the fibrous body the bundle was about 1 mm. in cross-section (Pl. 1, fig. 4), but on emerging it swelled out slightly and occasionally reached a diameter of 2 mm. One bundle in Curran's series even had a diameter of 3 mm.

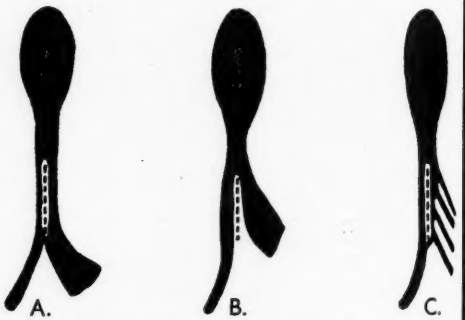
The right limb of the bundle varied greatly in size and in the ease with which it might be seen. In two hearts of the twenty it was visible in its whole length without dissection. In some cases it could be followed for about 1 cm. from the main bundle, in others for even less, and in four no sign of it could be found. In several specimens parts of the right limb were seen as white strands running in the moderator band or supraventricular crest; Pl. 1, fig. 3, is of a transverse section through the moderator band of heart no. 1 in which the right limb showed clearly without dissection.

The left limb was visible in four hearts as a broad band beneath the endocardium, and in three of these it was possible to remove the endocardium and trace the limb back to the main bundle. In most cases, however, the left limb could only be dissected by removing the pars membranacea septi; three types of left limb were thus demonstrated. These are shown in Text-fig. 2 and are as follows:

A. The text-book type arising from the bundle as it divides above the fleshy septum at the anterior limit of the membranous septum. It is shown in Pl. 1, fig. 2.

B. The limb arising from the bundle just after it leaves the fibrous body at the posterior margin of the membranous septum.

C. The limb arising by a number of small slips from the main bundle along its length between the posterior and anterior limits of the membranous septum.



Text-fig. 2. Diagram of the three types of left limb of the A.V. bundle. The dotted line represents the membranous septum and the node and bundle are figured as if exposed by removal of the membranous septum from above.

In a few cases it was possible to trace part of the left limb down the interventricular septum in continuity with the false tendons which cross the cavity of the left ventricle and act as bridges for the conduction of the specialized tissue to the free wall of the ventricle. Although the A.V. bundle was demonstrable in eighteen of the twenty hearts used, attempts to show the A.V. node by dissection succeeded in only five, the criteria being its position, its continuity with the A.V. bundle and its peculiar yellowish colour when fresh. When reference is made to the detailed histology of the node (Blair & Davies, 1935), it is not hard to understand the difficulty of delimiting this structure and rendering it visible to the naked eye. It was possible in these five cases to dissect the artery to the node, fully described by Gross (1921), and arising from it a small branch which followed the bundle through the fibrous body. It is seen in Pl. 1, fig. 4, in the centre of the bundle.

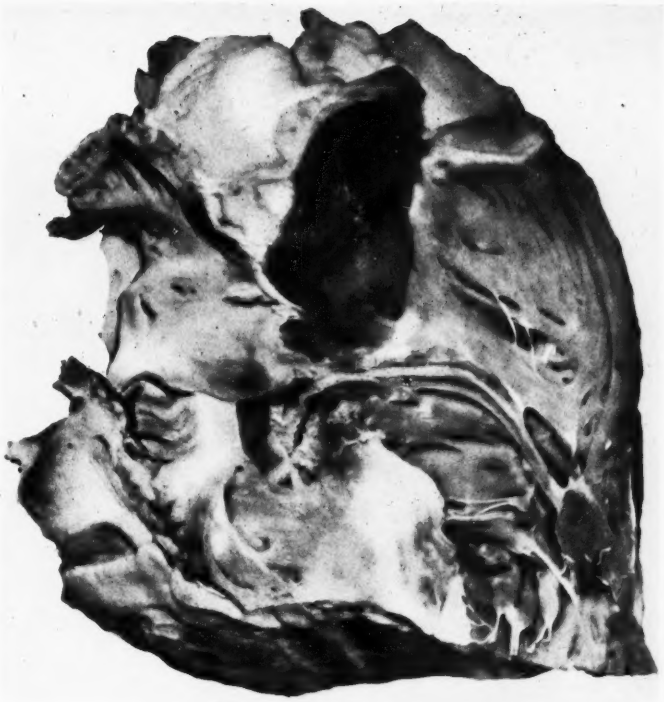


Fig. 1.

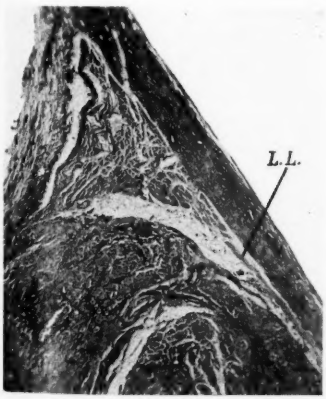


Fig. 2.

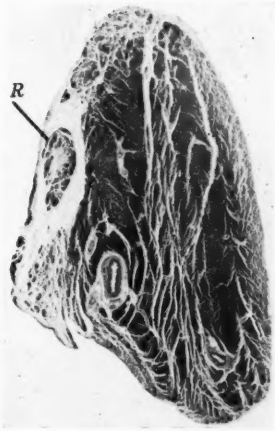


Fig. 3.

Fig. 4.

SUMMARY

1. Twenty human hearts were dissected to expose the A.V. node, the A.V. bundle and its limbs.

2. In eighteen hearts the bundle was found below the pars membranacea septi under the septal cusp of the tricuspid valve.

3. The right limb could be exposed in part in most of the hearts; in some, portions could be seen through the endocardium, and in two the entire length of the right limb stood out clearly without dissection.

4. The left limb was difficult to expose and varied in its method of origin.

5. The A.V. node was dissected as a demonstrable entity in five hearts in each of which the nodal artery from the right coronary was found.

I should like to express my indebtedness to Prof. C. M. West for helpful advice and to Mr A. Welch for technical assistance.

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EXPLANATION OF PLATE

(All figures are untouched)

Fig. 1. Heart no. 18 from the right side. Although the A.V. node and bundle can be identified in this specimen its main feature is the continuous sweep of the right limb via the moderator band to the anterior papillary muscle and free wall of the right ventricle. $\times \frac{7}{8}$.

Fig. 2. Longitudinal section through the membranous and fleshy septa to show the A.V. bundle dividing into right and left limbs. *L.L.* left limb. $\times 25$.

Fig. 3. Section of moderator band of heart no. 1 showing the right limb of the bundle lying just under the endocardium. This photomicrograph corresponds with the cut surface of the papillary muscle in fig. 1. *R.* right limb. $\times 15$.

Fig. 4. Section of the A.V. bundle in the fibrous body of the heart. *a*, branch to bundle from nodal artery. $\times 40$.

REGENERATION OF FIBRE DIAMETER AFTER CROSS-UNIONS OF VISCERAL AND SOMATIC NERVES

By S. A. SIMPSON and J. Z. YOUNG, *Department of Zoology and Comparative Anatomy, Oxford*

1. INTRODUCTION

In a normal nerve each function is subserved by fibres of a particular range of size. It is not clear to what extent functional efficiency is dependent on fibre diameter, but it seems certain that gross abnormalities following degeneration and regeneration, such as a great reduction in the size of the somatic motor fibres, must produce a corresponding functional derangement, even if the connexions are re-established correctly. Gutmann & Sanders (1943) have shown that after nerve suture the normal bimodal distribution of fibre sizes in the peroneal nerve of the rabbit is not restored, even after one year's regeneration. However, if the nerve is crushed, interrupting the axons but not the tubes in which they run, the bimodal distribution again begins to become apparent after about 200 days.

It is therefore important to discover the factors controlling the magnitude of fibre diameter attained after regeneration. Two obvious possibilities, which formed the basis of the present experiments, are: (1) that the diameter of each parent axon in the central stump determines that of the new fibre in the peripheral stump; (2) that the fibre diameter is determined by the size of the connective tissue tubes remaining in the peripheral stump after degeneration. Nageotte and Guyon (1918) and Sanders & Young (1944) have already shown by another method that there is some peripheral factor which affects the diameter of regenerated axons. They utilized the fact that the sciatic nerve contains motor, sensory, and sympathetic fibres, lying together in its upper portion, but disposed separately in its lower portion. After section and suture in the upper levels, the subsequent fibre connexions become mixed, so that fibres of all sizes grow down into branches some of which contained large, others only small, tubes. Yet after regeneration the fibres were found to be larger in the motor (large tube) than in the cutaneous (small tube) branches.

This condition could be attributed to restriction by the small tubes of the diameter attained by fibres growing within them. It was therefore attempted to settle the question by the more direct experiment of joining nerves containing fibres of different sizes. The necessary conditions were obtained by making unions between somatic and visceral (pre- and post-ganglionic) trunks. This results in the formation of very unusual terminal fibre connexions (see Langley & Anderson, 1904). It thereby became apparent that a further factor to be considered is that the diameter reached by a regenerating fibre is greatly influenced by the connexion which it effects peripherally. Somatic motor fibres which gain connexion with muscles become much larger than those which fail to make such connexion. Since this very important and interesting functional factor is operative it is evident that the possible constricting influence exercised by the tubes of the peripheral stump can be shown only by comparing nerves of different tube sizes but having comparable terminal connexions. Such comparison is possible in the present series of experiments, and shows that the restrictive effect of the peripheral tubes is marked only when these are very small in comparison with the size of the fibres in the central stump.

2. METHODS

The ideal method of testing the influence of central and of peripheral stumps on the size of regenerated fibres would be to join nerves containing, respectively, only large and only small fibres. Unfortunately, though there are nerves which approach this condition, all mammalian nerves with large fibres contain also some small ones. The following nerves in the rabbit, however, provide a close approach to the ideal conditions for such cross-unions:

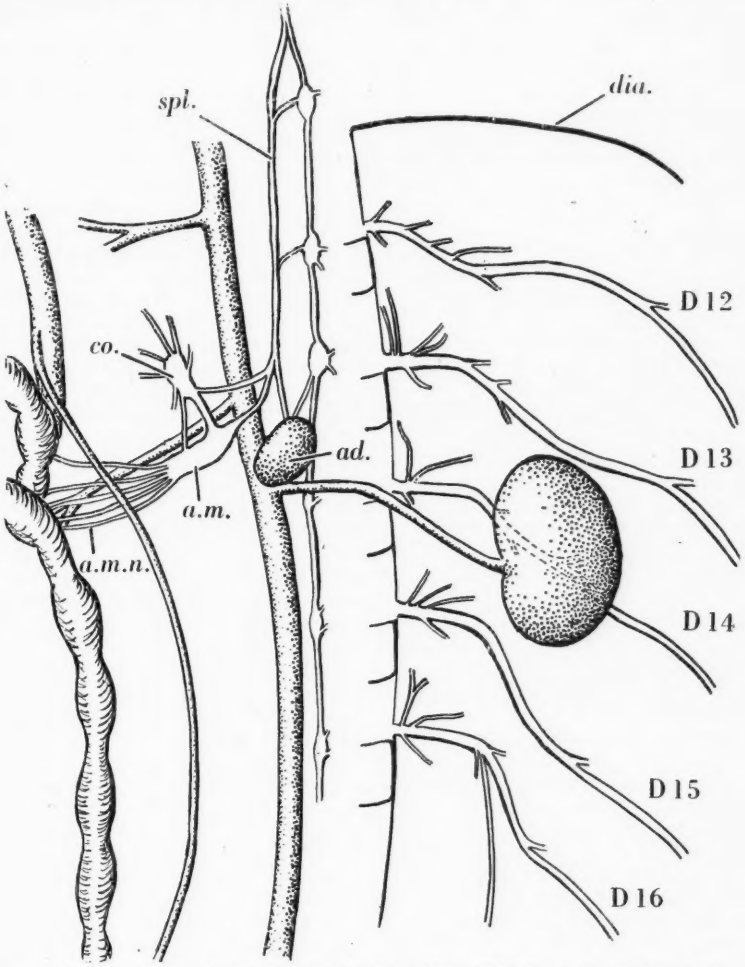
(1) The anterior mesenteric nerves, composed almost wholly of small non-medullated fibres.

(2) The great splanchnic nerve, composed mainly of small medullated fibres.

(3) The ventral rami of the post-thoracic spinal nerves, containing fibres of all sizes from the largest downwards, distributed bimodally.

Details of the composition of these nerves are discussed later. Their anatomical situation provides very many advantages for the making of cross-unions. Text-fig. 1 shows diagrammatically the arrangement of the solar plexus and neighbouring structures in the rabbit. The abdominal ganglia are highly variable: often there are separate coeliac and anterior mesenteric ganglia, as shown, but these may be almost fused, or a third ganglion may occur on the great splanchnic nerve. Notwithstanding such variations, the anterior mesenteric nerves form a sufficiently definite entity to be used in cross-

unions either as central or as peripheral stumps. The nerves never form a single bundle, but consist rather of a set of nearly parallel strands, running along the posterior aspect of the anterior mesenteric artery for a centimetre or more before breaking up into smaller branches. These strands therefore provide a most convenient source of non-medullated fibres, containing only a very few small medullated fibres. They are readily accessible at operation. If cut as far as possible from the ganglion they can be isolated as a definite trunk some millimetres long for use as a central stump. A cut made immediately distal to the anatomical ganglion isolates a trunk which can be used as a peripheral stump, although a few nerve cells are likely to be left peripheral to the cut, because of the diffuse nature of the ganglion.



Text-fig. 1. Diagram of solar plexus and related nerves in the rabbit. D12–16 are the post-thoracic somatic nerves. The great splanchnic nerve (*spl.*) divides into branches to the adrenal (*ad.*) and to the coeliac (*co.*) and anterior mesenteric (*a.m.*) ganglia. From the latter proceed the anterior mesenteric nerves (*a.m.n.*). *dia.* shows the position of the diaphragm.

The great splanchnic nerve leaves the sympathetic chain above the diaphragm and with practice can usually be found easily just behind that muscle. Thence it runs, as a single or double strand, backwards for as much as 2 cm. before dividing into branches to the ganglia of the solar plexus. A satisfactory length of nerve is therefore available for

suturing, but a serious handicap is its very small diameter, making it difficult to handle after being moved.

The ventral rami of the post-thoracic nerves (D12–D15) were used as a source of large fibres. Their correct naming presents difficulties: in the rabbit D12 is the last intercostal nerve; the first three lumbar nerves usually do not communicate to form a plexus, and the genito-femoral nerve arises from D16. Nerves D13 and D14 may therefore be called the anterior and posterior iliohypogastrics, and D15 the ilio-inguinal. However, attempts to adopt the nomenclature of human anatomy serve only to obscure the essential similarity of all these nerves. Each passes between the psoas muscles, giving branches thereto, then runs for a considerable distance across the ventral surface of m. obliquus and divides into two branches which penetrate and innervate the obliqui, finally emerging to innervate the belly skin.

The stretch of nerve across the mm. obliqui is easily isolated and is long enough to be joined to the anterior mesenteric or splanchnic nerves. Though convenient in this respect these post-intercostal nerves have the disadvantage that they give off small branches as they pass over the muscles, and the number of fibres in the nerves therefore differs slightly at different levels. There is also some possibility of anomalous results from escape of fibres from these branches after their severance.

Operative conditions and post-operative health of the animals employed provided no serious obstacles. Large animals in good health were used, irrespective of race or sex. Some were lost prematurely, but this was to be expected in such prolonged experiments, and there was little evidence of internal disturbance resulting from operation.

The selected nerves were cut and mobilized for a sufficient length and then joined by application of concentrated cockerel plasma (Young & Medawar, 1940). Without this plasma the experiments would have been difficult or impossible, since the nerve trunks are too small to allow of stitching without considerable interference: with the plasma good apposition was secured in most cases.

To investigate the apposition the actual site of union was always removed at autopsy, fixed and sectioned. The junctions were classified according to an arbitrary scale: 1 was used for a good junction, with minimal separation of the stumps; 2 indicated some separation, and hence a slight narrowing or waist; 3 was a bad union, with a definite gap of a millimetre or more. Control of conditions at the junction is most important in such experiments. Counts of the numbers and sizes of nerve fibres are significant only when considered in relation to the state of the union above them. Ideally, a detailed investigation should have been made of the variability introduced by this factor alone, but such a course would have meant a quite impracticable number of experiments of each type.

In most of the experiments several cross-unions were made in the one animal. Thus in rabbit 811, D12 was sutured into the peripheral stump of the great splanchnic, D13 into the anterior mesenteric, and D14, after severance, into its own peripheral stump. This procedure was economical of material and rendered comparison more reliable. Each piece of nerve fixed was identified by a small letter: thus 811*l* was the somatic nerve 10 mm. below the D14 union, and 811*j* a piece 2 cm. lower. (All pieces can be identified by reference to Table 1.)

A possible source of serious error is the invasion of the peripheral stump by fibres from other nerves severed during the operation. Powers of regeneration seem to be especially high in this part of the body (perhaps because of the high temperature), and in several experiments it was found that adventitious fibres had joined the peripheral stump from considerable distances. The most potent source of such invasion was the branches of the post-thoracic nerves to the psoas muscles (Text-fig. 1), especially as the point of operative union often lay directly upon these muscles, which may have been incised during nerve mobilization and have thus come to contain cut nerve ends. In some instances invasion of fibres had certainly occurred from such a source. Possible false conclusions from such connexions have therefore been carefully considered in each case, and the sections of the line of union examined with this in view. In future experiments of this type, wrapping, or some other form of nerve shielding, seems highly desirable.

The great length of somatic nerve necessarily mobilized to effect connexion with the splanchnic or anterior mesenteric nerves produced some complications. The long line of scarred muscle which resulted often brought about adhesions to the liver or stomach, so that post-experimental dissection was difficult. Within the nerve itself the mobilization delayed removal of the products of degeneration, so that numerous myelin remains were found at 100 and some even at 200 days after operation (Pl. 4, fig. 21). In this respect the condition of the nerves approached that of free nerve grafts. In some of the experiments actual grafts were made of one type of nerve into another, and the effects of long mobilization in direct unions were therefore an advantage in making the grafts more directly comparable with the cases of direct union.

All histological material was removed alive from the anaesthetized animal and stretched carefully on pieces of card. For counting of medullated fibres the nerves were fixed in Flemming's chrome-osmium-acetic mixture, embedded in paraffin through cedarwood oil, sectioned transversely at 4 μ, and stained with the Weigert method used by Gutmann & Sanders (1943). For study of non-medullated

Table 1

			Central stump																
			Somatic					Splanchnic						Anterior mesenteric				
		1	2	3	4	5	6	7	2	3	4	5	6	7	2	3	5	6	7
		Days of regeneration	Animal and nerve	Union	Level	No. of fibres	Area medullated %	% of medullated area >6 μ	Animal and nerve	Union	Level	No. of fibres	Area medullated %	% of medullated area >6 μ	Animal and nerve	Union	No. of fibres	Area medullated %	% of medullated area >6 μ
		—	999*c*	Normal nerve	—	2663	61·5	91	999*b*	Normal nerve	—	2192	48·2	4	732*a*	Normal nerve	14	0·1	0
		100	842*c*	Central stump	—	2180	38·6	65	—	—	—	—	—	—	—	—	—	—	—
Peripheral stump	Somatic	100	847*d*	1	Near	2256	33·3	59	959*f*	2	Near	1218	17·7	42	731*c*	1	11	0·1	0
			847*e*	—	Far	1487	16·7	27	959*h*	—	Far	1136	10·9	45	—	—	—	—	—
			841*a*	3	Near	1116	9·5	44	—	—	—	—	—	—	834*a*	2	19	0·1	0
			853*h*	2	Graft	2245	20·0	52	—	—	—	—	—	—	—	—	—	—	—
			859*b*	1	Graft	2795	28·4	59	—	—	—	—	—	—	854*a*	2	634	5·1	45
			842*a*	1	Graft	5158	26·6	28	—	—	—	—	—	—	959*a*	2	Many, not counted		
		200	811*l*	1	Near	3733	49·3	73	—	—	—	—	—	—	835*a*	2	43	0·3	0
			811*j*	—	Far	3240	46·5	72	—	—	—	—	—	—	958*a*	2	0	0	0
	Splanchnic	100	833*c*	2	Near	1306	14·0	40	854*e*	—	Near	350	6·6	5	848*g*	2	0	0	0
			859*f*	1–2	Graft	1493	22·8	64	—	—	—	—	—	—	—	—	—	—	—
			859*g*	—	Graft	1239	17·3	54	—	—	—	—	—	—	—	—	—	—	—
			853*c*	—	Graft	1914	20·0	59	—	—	—	—	—	—	—	—	—	—	—
		200	811*f*	1	Near	1433	41·0	65	—	—	—	—	—	—	—	—	—	—	—
	Anterior mesenteric	100	735*b*	2	Near	1160	4·1	20	841*d*	2	Near	69	0·1	0	853*m*	2	18	0·1	0
			833*a*	1	Near	Not counted	—	—	—	—	—	—	—	—	—	—	—	—	—
		200	811*b*	2	Near	2173	16·2	25	—	—	—	—	—	—	—	—	—	—	—

fibres, and usually for the site of union, Bodian's method was used, with alcohol-formol-acetic fixation.

3. COUNTING TECHNIQUE

It being surprisingly easy to draw false conclusions by simple visual or qualitative judgements of degree of medullation, resort was therefore made to counting and measurement. In most instances this was done by making direct photographs of the nerves by projection through the microscope on to bromide paper at a magnification of $\times 750$ (Sanders & Young, 1944). In a comparison of fibre sizes it is important that the degree of unavoidable error should be constant. This was ensured by the use of (1) standard histological technique, (2) repeated checking of magnification, (3) standard counting technique. In most cases not all the fibres in a nerve were measured, the sampling method used by Gutmann & Sanders (1943) being adopted. Fibres were referred to groups by their outside diameters, as in that method. Where fibres were not round, exact measurements were made of the longest diameter of the fibre and of that at right angles to the longest, the mean of the two measurements being taken. In the case of crenated fibres the diameter was measured and then 10 % of this was added (Duncan, 1934). Sampling technique was not used where the density of fibres appeared uneven; in such instances photographs of the whole nerve were taken, and all the fibres measured.

4. METHOD OF EXPRESSING RESULTS

Some difficulty is occasioned by the fact that it is not possible to test directly the effect of union of fibres of one size with those of another, because all the nerves used contain some range of fibre size. A means of expressing this range is therefore necessary, both in the normal nerves and in the results of cross-unions. Histograms or cumulative percentage curves of frequency of fibres of various diameters give good visual demonstration of the facts, but for numerical treatment we have used expressions involving the squares of the diameters (see Gutmann & Sanders, 1943). The general problem of the investigation may be stated as an attempt to ascertain to what extent the cross-sectional area presented by the tubes into which nerve fibres are growing affects the diameter which those fibres attain. We have therefore, for each of the normal nerves, calculated the area represented by fibres of various diameters. Since, however, the nerves differ widely in total area, it is necessary to divide the figures thus obtained by the total areas of the nerves, giving an *area factor*, or proportion of the total area which is occupied by fibres. Thus a normal somatic nerve (999*c*) has an area of fibre of 184,000 μ^2 and a total area of 299,000 μ^2, giving an area factor of 61·5 %. The great splanchnic (999*b*) with fibre area of 19,200 μ^2 and total area of 39,600 μ^2 has an area factor of 48·2 %.

Since the difference between the splanchnic and somatic nerves is, broadly, that the latter contain numerous fibres greater than 6 μ, we have also calculated the area occupied by fibres greater than this diameter. Thus in the above normal somatic nerve the fibres greater than 6 μ give an area of 166,000 μ^2, whereas for the great splanchnic nerve the figure is only 720 μ^2. In other words, for a given area the two nerves are occupied by similar areas of medullated fibre, but in the case of the somatic nerve the large fibres make up 91 %, and in the splanchnic nerve 4 % only of the medullated area.

5. NORMAL COMPOSITION OF THE NERVES USED

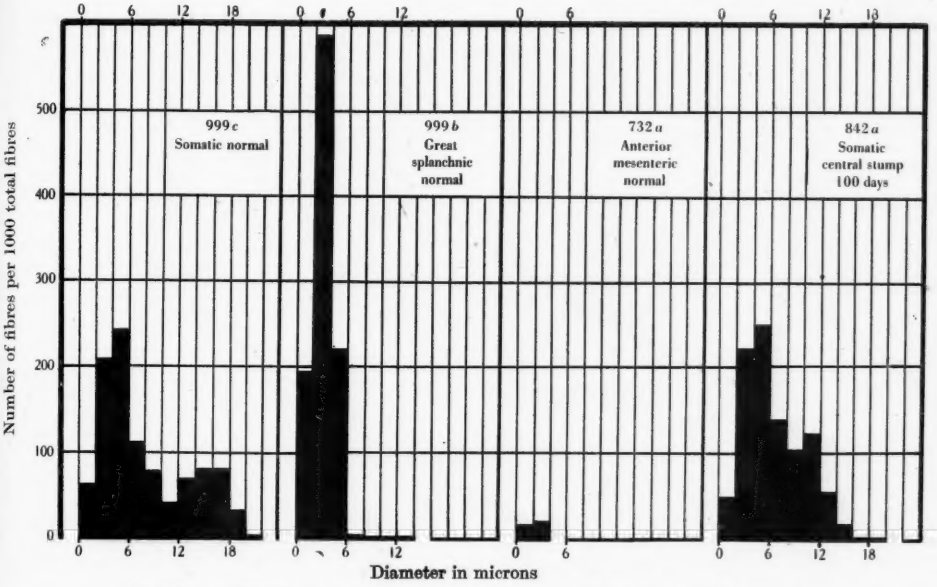
(1) *Nerves with large medullated fibres.* The ventral rami of the post-thoracic nerves contain a very high proportion of large fibres (Pl. 1, fig. 1). Text-figs. 2 and 4 show a histogram and a cumulative percentage curve of the distribution of fibres in the nerve (999*c*) at the middle of D12. This nerve contained 2663 medullated fibres with a sharply bimodal distribution similar to that found in other motor nerves. The group of large fibres constitutes a considerably greater proportion of the total than in the mixed region of the peroneal nerve recently investigated by Gutmann & Sanders (1943). They found 17·6 % of fibres of diameter greater than 8 μ, whereas the present post-thoracic nerves manifest 37·6 %. The area factor measure, already explained, shows that of the 61·5 % of the total nerve area occupied by medullated fibres 91 % is occupied by fibres of diameter greater than 6 μ. Moreover, the largest fibres present in these post-thoracic nerves are larger than those in the peroneal nerve.

We have not attempted to study the change in number of fibres along the course of any one nerve, nor to compare D12 with the other lumbar nerves used. The four nerves do not differ greatly in diameter, but they decrease slightly in size cranio-caudally. For purposes of experiment it would have been more satisfactory to use nerves containing large fibres only, but none such are known, and the lumbar nerves chosen, besides being conveniently situated, approach the ideal by reason of their high large-fibre content.

(2) *Nerves with small medullated fibres.* The great splanchnic nerve of the rabbit (Pl. 1, fig. 2) consists mainly of medullated fibres smaller than 6 μ in diameter. Nerve 999*b* manifested two trunks at the level studied, and these contained 2192 fibres distributed as shown in the histogram and cumulative percentage curves (Text-figs. 2, 4). The proportion (48·2 %) of the nerve area occupied by medullated fibres approaches that of a normal

somatic nerve, but 4% only of this area is composed of fibres larger than 6 μ. A similar composition with few large fibres has been noted by Bishop & Heinbecker (1930) and figured by Otuka (1940). The large medullated fibres are peculiar in having exceptionally thick sheaths.

(3) *Non-medullated nerves.* The anterior mesenteric nerves (Pl. 1, fig. 3) are a series of bundles composed almost wholly of non-medullated fibres, amongst which are scattered a very few very small medullated fibres (Text-fig. 2). Thus, in nerve 732*a* from the intervertebral foramen, turned backwards and united with the central end of D14, cut at some distance from its foramen: 100 days later a good union was found (Pl. 1, fig. 5), and histological examination confirmed that, despite some criss-crossing, there was no marked separation of the stumps. The peripheral stump (Pl. 1, fig. 4) 10 mm. below the union contained 2256 fibres: i.e. about the number in a normal nerve. The proportion of the nerve area (33·3%) occupied by medullated fibres was, however, much below normal, and of



Text-fig. 2. Histograms of composition of normal nerves, and of a central stump.

there were fourteen such fibres occupying less than 0·1% of the total area of the nerve. The average diameter of these fibres was 3·9 μ, and none was larger than 6 μ.

6. DIRECT UNION OF SOMATIC NERVES

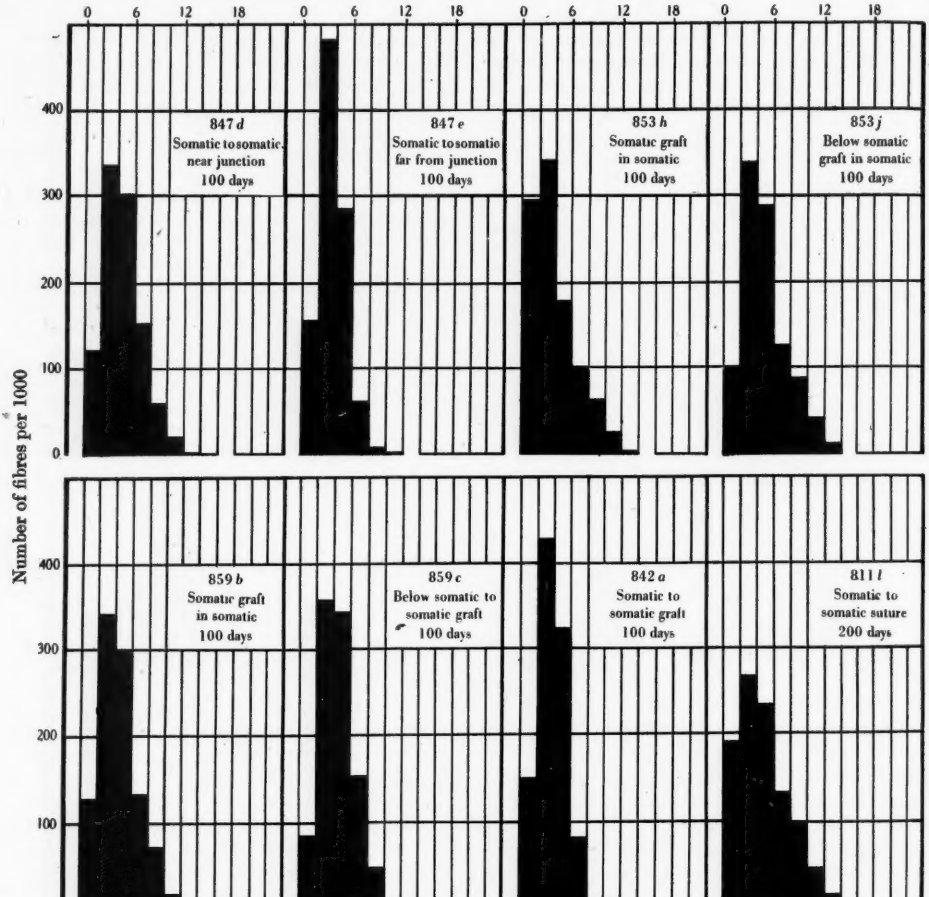
Before drawing conclusions about cross-unions it is essential to discover the precise fibre composition appearing after the suture of one lumbar nerve into another. In making the cross-unions, long stretches of nerve are freed from their surrounding tissue, a procedure inevitably resulting in considerable damage to the nerve, both from its handling and from interference with its blood supply. A similar nerve mobilization was therefore performed in most of the control sutures, the nerves generally being united with their neighbours.

The nerve 847*d* (D13) was cut close to its exit this 59% only was contributed by fibres of larger diameter than 6 μ. The largest fibre was 16 μ only in diameter, compared with the normal 22 μ. No bimodality appeared in the distribution (Text-fig. 3) and the peak of the small-fibre group was at 2–4 μ, instead of the normal 4–6 μ.

A second count made some 2 cm. distally on the same peripheral stump (847*e*) (Text-fig. 3) showed a somewhat smaller number of fibres, the result probably of loss of branches rather than of failure of medullation. However, the proportion (16·7%) of the nerve occupied by medullated fibres and the proportion (27%) of this contributed by fibres greater than 6 μ were both much smaller than at a point immediately below the suture, the conclusion being that increase of diameter and medullation were proceeding progressively along the distal trunk (see Gutmann & Sanders, 1943).

This case shows that 100 days after suture

medullation may be still far from complete. Later stages can be studied in 811*l*, where D14 was cut in such a way as to leave the two stumps held together by the epineurium, thus minimizing retraction. (This procedure favours regeneration in that the disturbances due to mobilization are avoided.) The nerves were removed 200 days later: longitudinal sections of the point of injury showed no marked separation of the stumps (Pl. 2, fig. 7): 10 mm. below this level (811*l*) there were 3733 medullated fibres distributed as shown in Text-figs. 3 and 4, and Pl. 1, fig. 6. The distribution shows no trace of bimodality, but the proportion of nerve area occupied by medullated fibres is greater than at 100 days after suture, and the proportion (73 %) of this area occupied by fibres greater than 6 μ is also greater, though it remains less than the corresponding proportion in a normal nerve. The largest fibres present remain smaller than those in a normal nerve. At 2 cm. below the injury (811*j*) the total fibre area and the area of fibres greater than 6 μ diameter are little less than at the more proximal level. If the diameters of the fibres are still increasing the gradient along the nerve is less steep than at the 100-days stage.



Text-fig. 3. Histograms of peripheral stumps after various direct unions between somatic central and peripheral stumps. Except where stated all counts taken 10 mm. below the union.

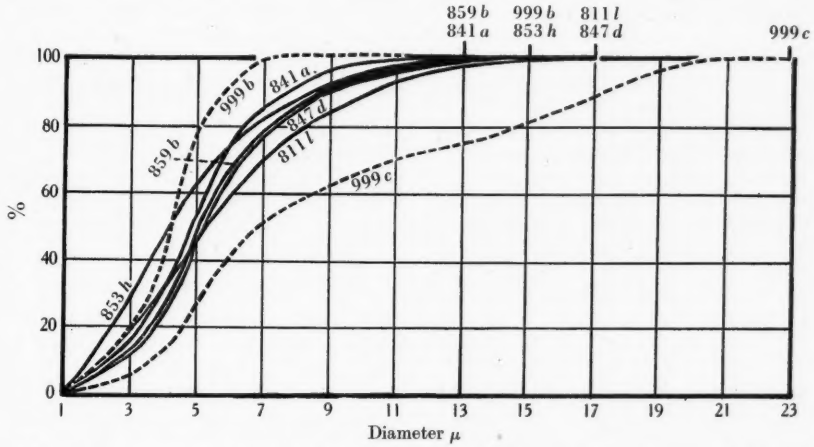
In a further case of suture, D12 as central stump

was joined to D13, but sections of the region of union examined after 100 days (841*a*) showed considerable separation: it was not surprising therefore to find only 1116 fibres, occupying 9·5 % of the nerve area, with 44 % only of the medullated area occupied by fibres larger than 6 μ (Text-fig. 4): 2 cm. distally there were still fewer and smaller fibres. This case shows how a bad union may affect the degree of medullation.

Some of the cross-unions to be discussed later were made by taking free grafts of nerves. As a control in three cases grafts were made from one lumbar nerve into another. In two of these cases (853*h* and 859*b*) in which 2 cm. of D15 was grafted to D14, the number and diameter of fibres were similar to those found after direct suture of one lumbar nerve into another (Text-figs. 3, 4). Since long sections of nerve were mobilized to make the sutures it was to be expected that they would behave much like grafts.

The third graft, 842*a* (Pl. 2, fig. 9), was a piece of D13 joined proximally to D12 but distally to a piece of great splanchnic, whose distal end, in turn, was joined to the anterior mesenteric, the ganglion being removed. When examined 100 days later, this series of grafts showed a progressive reduction, passing peripherally, in the number and size of medullated fibres. Many such fibres occurred in the grafted piece of somatic nerve, some in the splanchnic nerve, but very few in the anterior mesenteric nerve. It was noteworthy that all the fibres in the grafted somatic nerve were small and very numerous: the total of 5158 is more than twice that found in the central stump itself, 842*c* (Pl. 2, fig. 8, and Text-fig. 2), which contained 2180 fibres of the somewhat reduced diameter characteristic of central stumps (Gutmann & Sanders, 1943).

It is well known that much branching of fibres occurs after severance of a nerve, and that many new fibres penetrate each of the tubes of a peripheral stump (Cajal, 1928; Holmes & Young, 1942). However, in previous investigations it has been found that the number of medullated fibres is not greater in the peripheral than in the central stump, unless the latter is the smaller (Dogliotti, 1933; Aird & Naffziger, 1939). In the present case the characteristic features are the smallness of fibres (occupying 26·6 % only of the nerve, of which 28 % only is contributed by fibres greater than 6 μ), and the frequent occurrence of several fibres within a single tube (Pl. 2, fig. 9). Evidently an unusually large number of the fibres initially entering the peripheral tubes have become medullated. In seeking the reason for the difference between this case and that of the other somatic grafts the most obvious explanatory factor is the mode of peripheral connexion made by the fibres. In this case alone among the somatic peripheral stumps studied were the fibres unable to reach their normal end-organs. Recently it has been found (Sanders & Young, 1945) that, when the axons of the peroneal nerve are interrupted by crushing high up, while lower down the nerve is cut so that the outgrowing fibres cannot reach their end-organs, a phenomenon occurs exactly comparable to the one under discussion: many small fibres are found in the tubes between the crushed point and the neuroma which forms at the severed end of the nerve. Weiss & Taylor (1944) have also shown that connection with the periphery increases the size of regenerating fibres.



Text-fig. 4. Cumulative percentage curves to show results of simple suture of somatic nerves: 847*d*, 100 days after good union; 841*a*, 100 days after poor union; 811*l*, 200 days after good union; 853*h* and 859*b*, after grafting somatic nerves. 999*b* and *c* normal splanchnic and somatic nerves. The end of each curve is marked along the top line.

A most important fact is thus revealed, that when regenerating fibres succeed in making peripheral contact they increase in diameter throughout their whole length, at the expense of others in the same tube. The forces which produce this effect are still unknown, but they must constitute some of the most important of those controlling regeneration.

Summary of effects of direct union of somatic nerves

The results give a reasonably consistent picture of the progress of medullation after simple union or after grafting of somatic nerve, which is summarized in the cumulative percentage curves of Text-fig. 4. The curves are approximately similar in the various regenerated nerves and show that the proportion of the larger fibres present is less than that in a normal somatic nerve but much greater than that in a normal splanchnic nerve. If union has been good and connexion made with the periphery, 100 days after operation the largest fibres 10 mm. below the union may be some 16 μ in diameter and about 60 % of the total medullated fibre area made up by fibres greater than 6 μ: there is a considerable decline in diameter passing peripherally along the nerve. However, 200 days after the operation the fibres are both more numerous and larger, more than 70 % of the area of medullation being made up by fibres above 6 μ, and the decline of fibre diameter along the distal stump being less steep: the nerve remains subnormal in condition, in particular without any reappearance of bimodal distribution.

7. DIRECT UNION OF SPLANCHNIC NERVE

Before assessing the results of cross-unions it is desirable to know the fibre composition resulting in the peripheral stump after the great splanchnic nerve has been cut and the ends rejoined. The nerve being small, the operation is difficult, and we obtained but one satisfactory experiment, 854*e*, in which the nerve was first cut almost completely and the remaining strand then crushed thoroughly. This procedure should have provided optimal conditions for regeneration, but unfortunately no longitudinal sections were prepared to show conditions at the point of injury. However, sections of the peripheral stump showed the presence, 100 days after operation, of 350 medullated fibres occupying 6·6 % of the total area of the nerve: only 5 % of this medullated area was made up of fibres greater than 6 μ, whilst the largest fibre in the nerve was 10 μ only in diameter (Pl. 2, fig. 10). From the uncertain nature of the union in this case we cannot be sure that it represents a typical result of simple great splanchnic regeneration, but it suggests that, as would be expected, small fibres only are regenerated.

8. DIRECT UNION OF ANTERIOR MESENTERIC NERVES

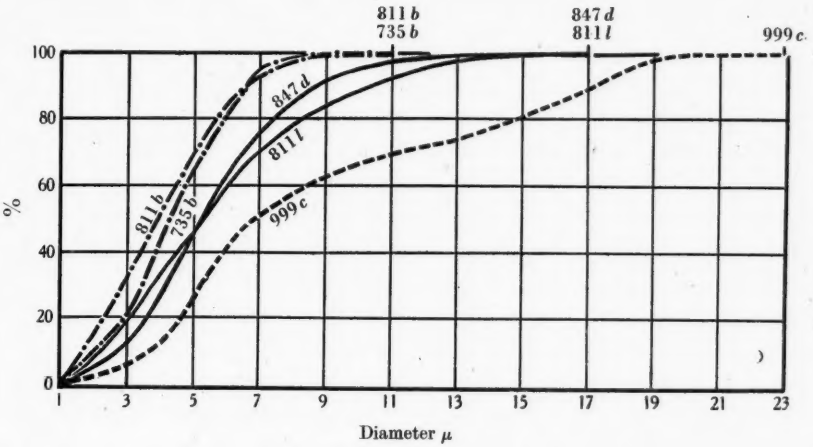
The anterior mesenteric nerves are such irregular bundles that it was decided not to attempt to sever them completely and to make a union. Instead, a single cut was made across the greater part of the bundle, just distal to the ganglion. No great retraction occurred, and when the site was examined 100 days later there was no obvious swelling or other sign of disturbance. Longitudinal sections showed that the cut had been made through the distal part of the ganglion, leaving a few nerve cells on its peripheral side. This, together with the fact that not all of the bundles were severed, means that some of the fibres had not undergone any interruption, although undoubtedly the majority had done so. The cross-sections of the distal stump 853*m* (Pl. 2, fig. 11), stained by Weigert's technique, showed bundles very similar to those of the normal anterior mesenteric nerves, containing in all eighteen medullated fibres, occupying less than 0·1 % of the area of the nerve. The average diameter of these fibres was 1·5 μ only and none was larger than 6 μ.

9. UNION OF LARGE CENTRAL FIBRES WITH SMALL PERIPHERAL TUBES

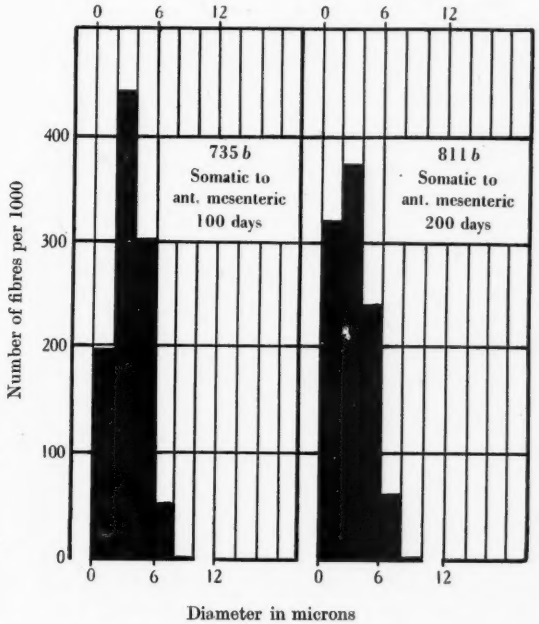
(1) *Union of somatic with anterior mesenteric nerves.* This most interesting union was made successfully in three cases, all of which agree in demonstrating that medullated fibres can be produced in a non-medullated post-ganglionic nerve, but that such medullated fibres are smaller than those formed when large fibres grow into large tubes.

In 735*b* the anterior mesenteric nerves were cut distal to the ganglion, and sutured to the mobilized central end of D12. The ganglion itself was left in place, and sections of the material removed 100 days later showed the somatic nerve running past a large mass of nerve cells into a region of mixed nerve cells and bundles, from which the main anterior mesenteric nerves arose. There was slight separation of the stumps, varying for the different bundles: some were so close to the central stump as to constitute a group 1 union, others showed a separation classed as a group 2 union.

The bundles in the peripheral stump were well filled with small medullated fibres (Pl. 3, fig. 14). In all the bundles included in the cross-section there were 1160 fibres, but these were mostly small and occupied 4·1 % only of the nerve area: moreover, few of them were larger than 6 μ, representing 20 % only of the total medullated area (Text-figs. 5, 6). Since there are normally very few medullated fibres in this nerve it is evident that nearly all those found therein had formed in Schwann tubes previously devoid of them. Presumably the myelin is formed in the usual manner in conjunction with Schwann



Text-fig. 5. Cumulative percentage curves to show small diameter attained in the peripheral stump by fibres following suture of somatic into anterior mesenteric nerves: 735*b*, after 100 days; 811*b*, after 200 days. 847*d* and 811*l* show peripheral stumps 100 and 200 days after direct somatic-somatic suture. 999*c*, normal somatic nerve.



Text-fig. 6. Histograms of distribution of medullated fibres in anterior mesenteric nerves after union with somatic nerves.

cells. The most likely supposition is that the 'cells of Remak' of the typical non-medullated fibre, presumably of similar origin to Schwann cells, when supplied, *inter alia*, with axons of suitable diameter, can assist in myelin formation. Another possibility is the migration of the Schwann cells from the central stump, but this is very unlikely because the stretch of medullated nerve examined was at least 1 cm. long, and Schwann cells are probably not very actively migratory when in contact with an axon.

Rabbit 833*a* showed a similar state of affairs when examined 100 days after suture. In this specimen the anterior mesenteric nerves were cut farther from the ganglion, which was almost completely removed. Longitudinal sections confirmed excellent union with the anterior mesenteric bundles (Pl. 2, fig. 12), which were full of medullated fibres. The bundles became curled in fixation and no satisfactory transverse sections could be obtained for counting; their general composition, however, was evidently very similar to that in 735*b*, none of the fibres being larger than 6 μ. The union was here so good that the small size of the new fibres cannot be ascribed to any difficulty in crossing from one stump to the other.

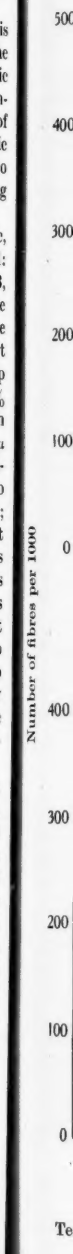
These two instances show that the large fibres can produce medullated fibres when joined with the tubes of even very small non-medullated nerves. The restriction on diameter increase persists during the second 3 months after union. Nerve 811*b* was examined 200 days after a successful union of D13 with the anterior mesenteric nerves, the ganglion being turned aside. The union was not quite so close as in 833*a*, and was graded as 2: the bundles in the peripheral stump contained a total of 2173 small medullated fibres, little larger than those encountered after 100 days' regeneration. They occupied 16·2 % of the nerve area, and only 25 % of this was made up of fibres larger than 6 μ, the largest fibres being 10 μ only in diameter. These figures may be compared with those for the direct suture of somatic with somatic nerve (811*l*) and of somatic with splanchnic (811*f*), made in the same animal (Text-fig. 9). In seeking the factor responsible for this limitation of diameter it must be remembered that in the cross-unions of somatic into visceral nerves the fibres effect no normal functional connexions at the periphery, and that there is evidence that, in the absence of such connexions, the rate of increase of fibre diameter is low. The greater size of the fibres in 811*l* than in the other two nerves may be partly due to this factor. However, the somatic fibres in the anterior mesenteric, 811*b*, are smaller than those in the splanchnic, 811*f*, though in neither of these nerves have they normal peripheral connexions. Similarly, they are smaller in 735*b*, an anterior mesenteric peripheral stump, than in 833*c* or 842*a*, splanchnic and somatic, which have also received somatic fibres for 100 days without permitting these to reach normal peripheral connexions. There is thus clear evidence that the small size of the tubes in the anterior mesenteric nerves exercises a restrictive influence on the increase in diameter of regenerating fibres within them, irrespective of the terminal connexions.

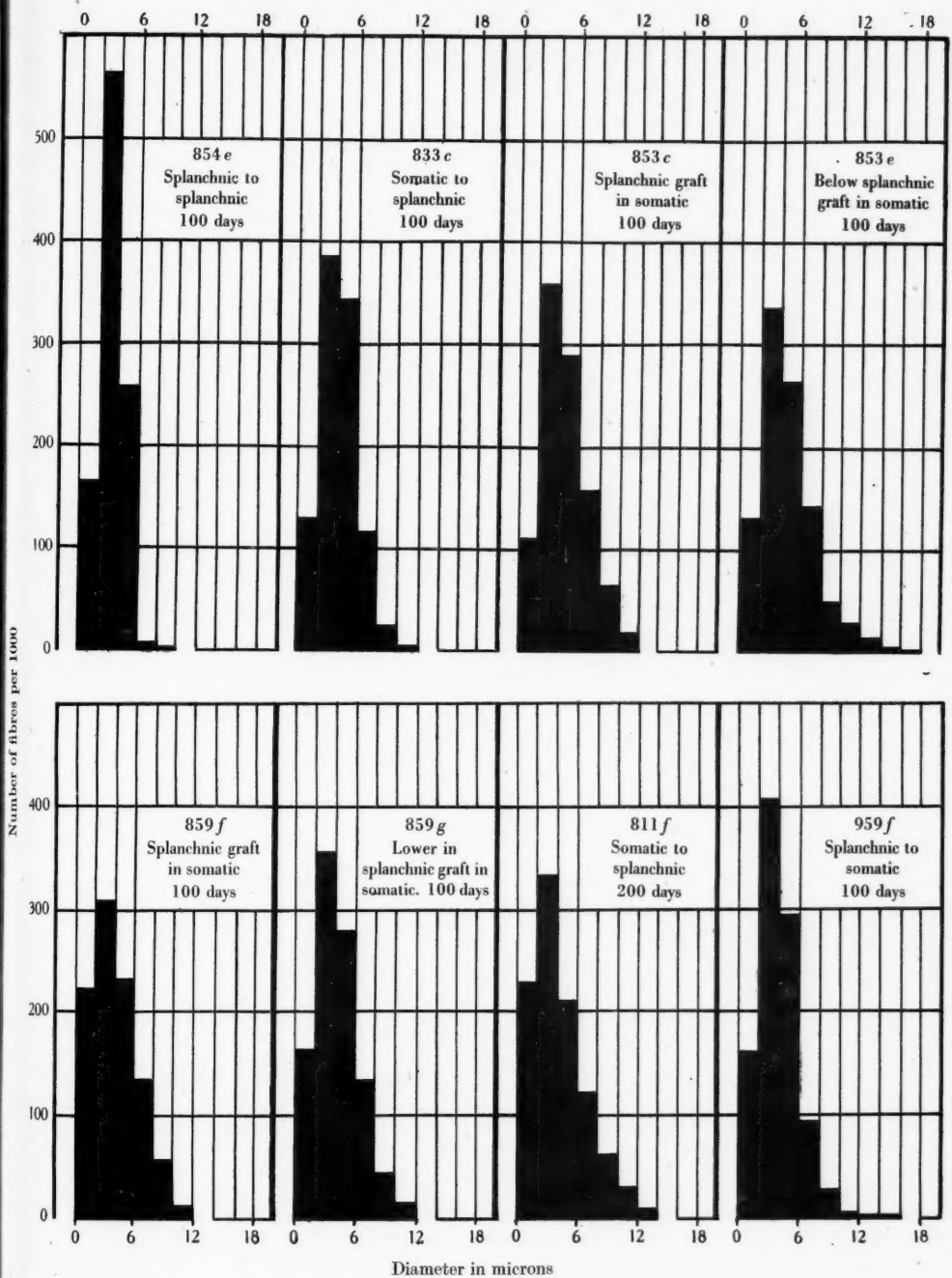
(2) *Union of somatic with splanchnic nerves.* This cross-union was made in one animal leaving the ganglion attached to the lower end of the splanchnic nerve, and in another animal after partial ganglionectomy. In two further animals, pieces of splanchnic nerve were used as grafts in a somatic nerve, thus allowing the regenerating fibres to establish normal peripheral connexions after passing through the graft.

In 833*c*, nerve D13 was joined to the splanchnic, and the main solar plexus ganglia were removed: union was found to be moderate, grade 2 (Pl. 3, fig. 17). The central stump being larger than the peripheral, some of the fibres emerging from the former made accessory bundles extending a short distance along the latter. The peripheral stump contained 1306 medullated fibres, occupying 14·0 % of the nerve area: the largest fibre was 12 μ only in diameter, and the area of those greater than 6 μ was 40 % only of the total medullated area (Text-figs. 7, 8, and Pl. 3, fig. 16). It was not possible to determine the peripheral connexions of these fibres; presumably most ended blindly at the lower cut end of the splanchnic nerve, whilst others perhaps reached the adrenal. Evidently the somatic fibres had produced in the splanchnic nerve new fibres considerably larger than those normally resident there. Though these new fibres were not nearly so large as those found in a somatic peripheral stump connected with the periphery (847*d*) they were only little smaller than the fibres found in the somatic stump 842*a*, which was not allowed to make connexion with the normal end-organs.

Examination was made of 811*f* 200 days after a very good union of D12 with the splanchnic nerve, the ganglion being left intact: 1433 medullated fibres (considerably larger than those of 833*c*) were present, occupying 41·0 % of the nerve; 65 % of the medullated area was made up of fibres larger than 6 μ, the largest being 14 μ. The connexions made by these fibres in the solar plexus and the adrenal remain uncertain. The fact that the fibres were smaller than those in the somatic peripheral stump 811*l* (see Text-fig. 9) may have been due partly to the influence of the peripheral connexions, partly to the constricting influence of the small splanchnic tubes.

The two instances of grafted great splanchnic nerve show very clearly that large fibres can be formed in even small tubes. The more successful was 859*f*, placed in D13: apposition was good at the upper junction for most of the bundles, and the union was classed as 1–2. The great majority of the



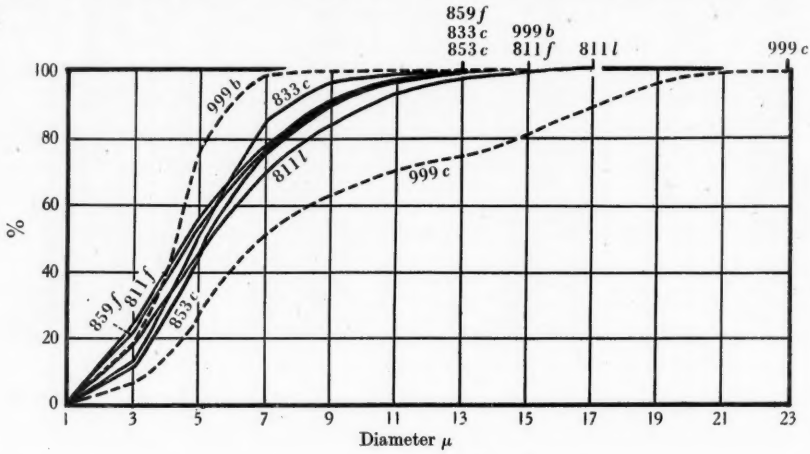


Text-fig. 7. Histograms of peripheral stumps 10 mm. below cross-unions involving the great splanchnic nerves.

fibres ran within the graft, but a few bundles extended some distance alongside it: such accessory bundles became scarcer distally, and few extended the whole 15 mm. length of the graft. Counts made at two levels in the graft showed 1493 and 1239 fibres respectively with nerve areas 22·8 and 17·3 %, 64 and 54 % being made up by fibres greater than 6 μ. (The distributions are shown in Text-figs. 7 and 10.) Comparison with the figures for 859*b*, in the same animal, where a piece of somatic nerve was used as the graft, shows no very marked differences. Moreover, the fibres are considerably larger than those of 833*c*, a piece of splanchnic nerve containing somatic fibres not connected with by a somatic central stump shows the influence of the central stump upon regeneration. On the other hand, the fewness of medullated fibres compared with the number produced in 854*e* by innervation by the splanchnic of its own peripheral stump shows again the restrictive effect of the peripheral tubes, although it must be remembered that the terminal connexions are also different in the two cases.

10. SMALL FIBRES GROWING INTO LARGE TUBES

(1) *Union of great splanchnic with a somatic nerve.* The actual operation was not difficult, but a serious



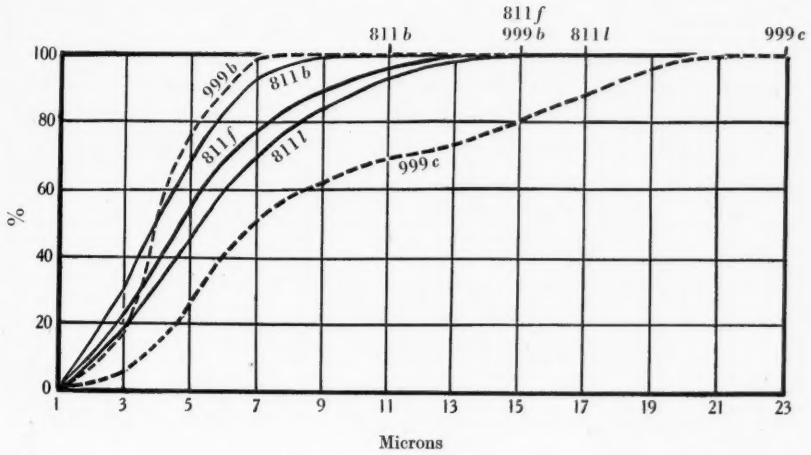
Text-fig. 8. Cumulative percentage curves to show distribution of fibres 10 mm. below union of somatic into splanchnic nerves. 833*c*, 100 days after the union. 859*f* and 853*c*, 100 days after grafting pieces of great splanchnic into a somatic nerve. 811*f*, 200 days after union. 999*b* and *c*, normal splanchnic and somatic nerves for comparison.

their normal end-organs. Evidently therefore the tubes of 859*f* have become dilated under the influence of the large somatic fibres having normal peripheral connexions. This result was confirmed in the second grafted piece of great splanchnic nerve, 853*c*, though in this case the closeness of the union was not studied. The figures in Table 1 and in Pl. 3, fig. 18, show that the fibres present in this graft were similar to those in a piece of somatic nerve (853*h*) used as a graft in the same animal (Text-fig. 10).

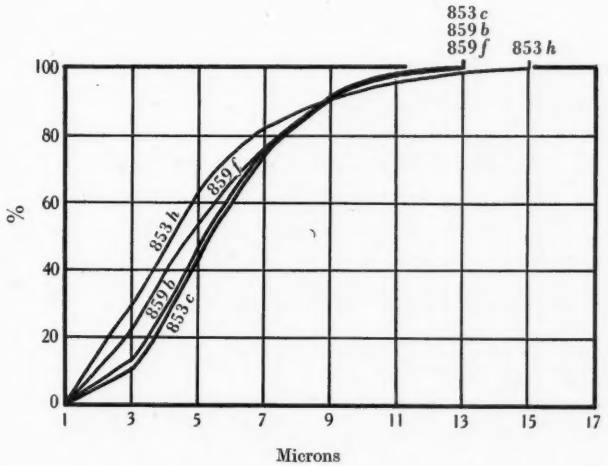
(3) *Union of splanchnic with anterior mesenteric nerves.* The one experiment (841*d*) in which this union was made produced sixty-nine small medullated fibres in the peripheral stump after 100 days: i.e. a number definitely in excess of that normally found in the anterior mesenteric, but much less than that obtaining in the splanchnic. The occurrence of fewer medullated fibres after this experiment than after innervation of the anterior mesenteric source of error arose from the point of union lying on the psoas muscles, which contain bundles of nerve fibres. The peripheral stump being larger than the central, there was consequent opportunity for complicating fibres from extraneous somatic nerves to invade the union. In one case such invasion certainly took place and the experiment was disregarded.

In a second case, 959*f*, examined after 100 days, a good union of splanchnic central and somatic peripheral stumps had been made and undoubtedly most of the fibres in the latter came from the former. However, muscle containing nerve fibres surrounded the point of union: some of these fibres were proceeding from the somatic nerve to the muscle, but the possibility that others were passing in the opposite direction could not be excluded.

Transverse sections (Pl. 4, fig. 20) showed larger fibres in the peripheral than in the central stump. The former contained, 10 mm. below the junction,



Text-fig. 9. Cumulative percentage curves of distribution of fibre sizes 10 mm. below union of somatic nerves into anterior mesenteric (811*b*), great splanchnic (811*f*) and somatic nerves (811*l*) 200 days after operation. 999*b* and *c*, normal splanchnic and somatic nerves for comparison.



Text-fig. 10. Cumulative percentage curves of distribution of fibres in grafts 853*h* and 859*b* of somatic into somatic nerves, 853*c* and 859*f* of splanchnic into somatic nerves. 100 days after operation. No clear effect of tube size on regeneration is apparent.

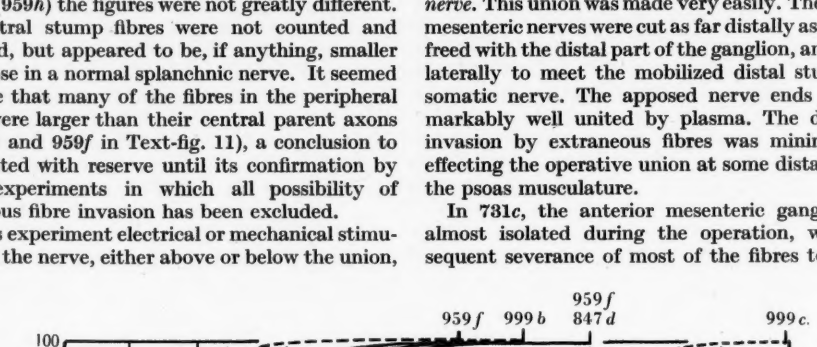
1218 fibres, having a myelin area representing 17·7 % of the total nerve area, 42 % of this myelin area being made up by fibres larger than 6 μ. More distally (959*h*) the figures were not greatly different. The central stump fibres were not counted and measured, but appeared to be, if anything, smaller than those in a normal splanchnic nerve. It seemed therefore that many of the fibres in the peripheral stump were larger than their central parent axons (cf. 999*b* and 959*f* in Text-fig. 11), a conclusion to be accepted with reserve until its confirmation by future experiments in which all possibility of extraneous fibre invasion has been excluded.

In this experiment electrical or mechanical stimulation of the nerve, either above or below the union, caused contraction of the body-wall muscles. The threshold was higher above the union, as would be expected *ceteris paribus* if the fibres there were smaller. This result agrees with that of Langley & Anderson (1904), who recorded contraction of the thyro-arytenoid muscle after suture of the preganglionic fibres of the cervical sympathetic into the peripheral stump of the recurrent laryngeal nerve, and of the sterno-mastoid muscle after a similar suture of the cervical sympathetic into the spinal accessory nerve.

A further case, in which the diameter of fibres appeared to be greater in the peripheral than in the central stump, was revealed by measurements made of the peripheral stump below the graft of a piece of great splanchnic nerve into a somatic nerve. In Text-fig. 7 the histogram of 853*e*, from below the graft, shows fibres larger than those in 853*c* within it. Here again, however, the possibility of invasion of the lower junction by somatic fibres cannot be altogether excluded.

(2) *Union of anterior mesenteric with a somatic nerve.* This union was made very easily. The anterior mesenteric nerves were cut as far distally as possible, freed with the distal part of the ganglion, and turned laterally to meet the mobilized distal stump of a somatic nerve. The apposed nerve ends were remarkably well united by plasma. The danger of invasion by extraneous fibres was minimized by effecting the operative union at some distance from the psoas musculature.

In 731*c*, the anterior mesenteric ganglion was almost isolated during the operation, with consequent severance of most of the fibres to it from



Text-fig. 11. Cumulative percentage curves of distribution of fibres after union of splanchnic with somatic (959*f*), somatic with splanchnic (859*f*) and somatic with somatic nerves (847*d*). All after 100 days. 999*b* and *c* normal splanchnic and somatic nerves.

the splanchnic nerves: union between the anterior mesenteric nerves and D 13 was very close. Examined 100 days after union, the peripheral stump contained eleven very small medullated fibres only, representing less than 0·1 % of the area of the whole nerve. Yet successful union was proved by the presence of very many non-medullated axons in each of the tubes of the peripheral stump.

An essentially similar result after 100 days was given by 834*a*, despite a union not so satisfactory: the peripheral stump contained nineteen small medullated, and very many non-medullated, fibres (Pl. 4, fig. 21).

In two further cases (854*a*, 959*a*) the union had made contact with the traumatized muscles and medullated fibres were found, in one case as a single bundle, in the other scattered throughout the peripheral stump (see Table 1).

Anterior mesenteric fibres made to grow into

somatic nerves were in two animals left to regenerate for 200 days: in one of these animals very few, and in the other no, medullated fibres appeared, yet the unions were moderately well made and non-medullated fibres were abundant in the peripheral stumps of both (Pl. 4, fig. 23). These fibres seemed slightly larger than those seen after 100 days, but they are too variable to confirm this impression by measurement. Certainly fibres of the anterior mesenteric nerve are not able to increase in diameter even when made to grow into large tubes: they remain as numerous small fibres mostly in contact with the walls of the host tubes.

In all these experiments the nerves were stimulated electrically and mechanically before fixation. Only in the cases in which invasion from somatic motor fibres had occurred (854*a*, 959*a*) did this produce contraction of the mm. obliqui. The inability of the abundant non-medullated post-ganglionic fibres present in the other nerves to establish functional connexion with the muscle fibres agrees with the finding of Langley & Anderson (1904) that post-ganglionic cervical sympathetic fibres after union with the hypoglossal nerve were unable to cause contraction of the tongue. Unfortunately, these experiments are insufficient of themselves to determine whether this failure to establish muscle connexion is due to some qualitative difference between different fibre types or simply to the very small size of the post-ganglionic fibres.

(3) *Union of anterior mesenteric with great splanchnic.* In one experiment (848*g*) the anterior mesenteric fibres were made to grow into the great splanchnic nerve, thus making a circle in which the processes of the ganglion cells returned to those cells. As in the last experiment, the anterior mesenteric fibres grew out abundantly but failed to become medullated. The endings made by the fibres when in contact with their own cell bodies have not yet been studied.

DISCUSSION

The results here submitted show that the control of fibre diameter in a regenerating nerve is exercised by at least three factors: (1) the diameter of the central fibres, (2) the nature of the terminal fibre connexions made, (3) the size of the peripheral tubes. It is possible that a fourth factor, not investigated here, is the length of nerve to be regenerated. The influence of the central stump is well shown by the fact that somatic fibres growing into post-ganglionic trunks, previously non-medullated, can produce therein medullated fibres. Further, the larger somatic fibres are able, when growing into a splanchnic nerve, to produce in it fibres larger than are normally present, even without effecting typical peripheral connexions: this result is similar to one obtained by Langley & Anderson (1904), who found after union of cervical somatic nerves with the superior cervical sympathetic trunk that the latter came to contain fibres larger than are normally found therein.

The converse effect of the central stump is shown by the inability of splanchnic nerve fibres to make, in the large tubes of a somatic nerve, fibres equivalent in size to those produced by regenerating somatic fibres (Text-fig. 11). An even more striking but comparable phenomenon is the inability of the post-ganglionic fibres of the anterior mesenteric ganglion to produce medullated fibres when growing into the large tubes of a somatic nerve. The difference in behaviour between the processes of sympathetic cells and those of somatic sensory and motor cells may be associated with the multiplicity of branches of the sympathetic cell, or with some qualitative difference of the axoplasm.

That the peripheral connexion exerts an influence on the diameter of the fibres in the regenerating nerve appears clearly from the present experiments. If a somatic nerve is sutured and also cut peripherally so that the regenerating fibres cannot reach their end-organs the fibres in that nerve will be more numerous and smaller than after simple suture. In such a nerve with an obstructed lower end many small fibres occur in each tube and evidently an unusually large number of the initial invading sprouts become medullated. The unions between somatic central and splanchnic peripheral stumps provide a very satisfactory confirmation of this effect. Where the somatic fibres grow directly into the splanchnic nerve, left *in situ*, they produce fibres smaller than those found when a piece of splanchnic nerve is used as a graft in a somatic nerve, the fibres in the latter case being able to reach normal peripheral endings. Very similar effects of the peripheral influence on medullation have been found in the nerves of the hind limb (Sanders & Young, 1945). It remains to discover how this influence is exerted.

Since the terminal connexions affect the diameters attained by regenerating fibres it is possible to assess the degree of restriction exercised by the small-sized peripheral tubes only by comparing nerves which end similarly. Although the experiments were not planned with the possibility of a terminal influence in mind, they provided several suitably comparable cases, which demonstrate that the small tubes of the anterior mesenteric nerves restrict the growth of somatic fibres within them. There is therefore good evidence that very small tubes, such as those of a non-medullated nerve, restrict the growth of regenerating fibres. On the contrary, the pieces of splanchnic nerve used as grafts in a somatic nerve, in such a way that the fibres were enabled to reach the periphery, came to contain fibres nearly, or quite, as large as those occurring in pieces of somatic nerve similarly

grafted. The restrictive influence of moderate-sized tubes is therefore not severe. Though further evidence on this point is desirable, the indications are that, in man, a piece of cutaneous nerve used as a graft in a motor nerve would not unduly restrict the growth of the motor fibres. Any restrictive influence might also be of practical importance in connexion with the shrinkage of the tubes of a peripheral stump left for long without innervation. Holmes & Young (1942) and Sanders & Young (1944) found evidence of delay in medullation under such conditions; possibly the extra collagen developed in the tube walls limits their extensibility. The tubes in the present experiments were refilled within a few weeks of degeneration.

The limiting effect of small tubes on regeneration is shown by the thinness of the myelin developed. All measurements so far given have represented the outside diameter of fibres. However, comparison of Pl. 1, fig. 6 with Pl. 2, fig. 15 and Pl. 3, fig. 19, shows that when large fibres inflate small tubes they produce a myelin sheath which is thinner than that of a fibre of similar *total* diameter produced in a larger tube. We have not yet made measurements to study this effect further, or to discover whether terminal connexion affects myelin thickness. Clearly such inquiry is of the greatest importance in analysing the restrictive influence of the tube, and in elucidating the nature of the myelin sheath and the factors controlling its thickness.

SUMMARY

1. The diameter reached by regenerating nerve fibres depends on their effecting connexion with their end-organs. Nerves without terminal connexions become filled with many and small nerve fibres.

2. When fibres grow out from somatic nerves into the non-medullated anterior mesenteric nerves, medullated fibres are formed in the latter. Such fibres are, however, of smaller diameter and have thinner myelin than those formed after union of a somatic with a somatic or a splanchnic nerve.

3. When a somatic nerve, containing large medullated fibres, is united with the splanchnic nerve, containing small medullated fibres, the new fibres produced in the splanchnic nerve are of diameter similar to that produced in a somatic peripheral stump with similar terminal connexions. The large central fibres can therefore inflate the smaller peripheral tubes.

4. The size of new fibres is therefore controlled partly by the size of their parent fibres, but very small Schwann tubes in the peripheral stump have a restrictive influence on fibre growth.

5. The non-medullated fibres of the anterior mesenteric nerves do not produce medullated fibres when made to grow into the Schwann tubes of a somatic nerve.

This research was assisted by a grant from the Rockefeller Foundation. We are grateful to Dr F. K. Sanders for reading and criticizing the manuscript, to Dr H. Motz for discussion of the statistical problems involved, and to Mr P. B. Medawar for preparing the plasma used in the experiments.

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EXPLANATION OF PLATES

PLATE 1

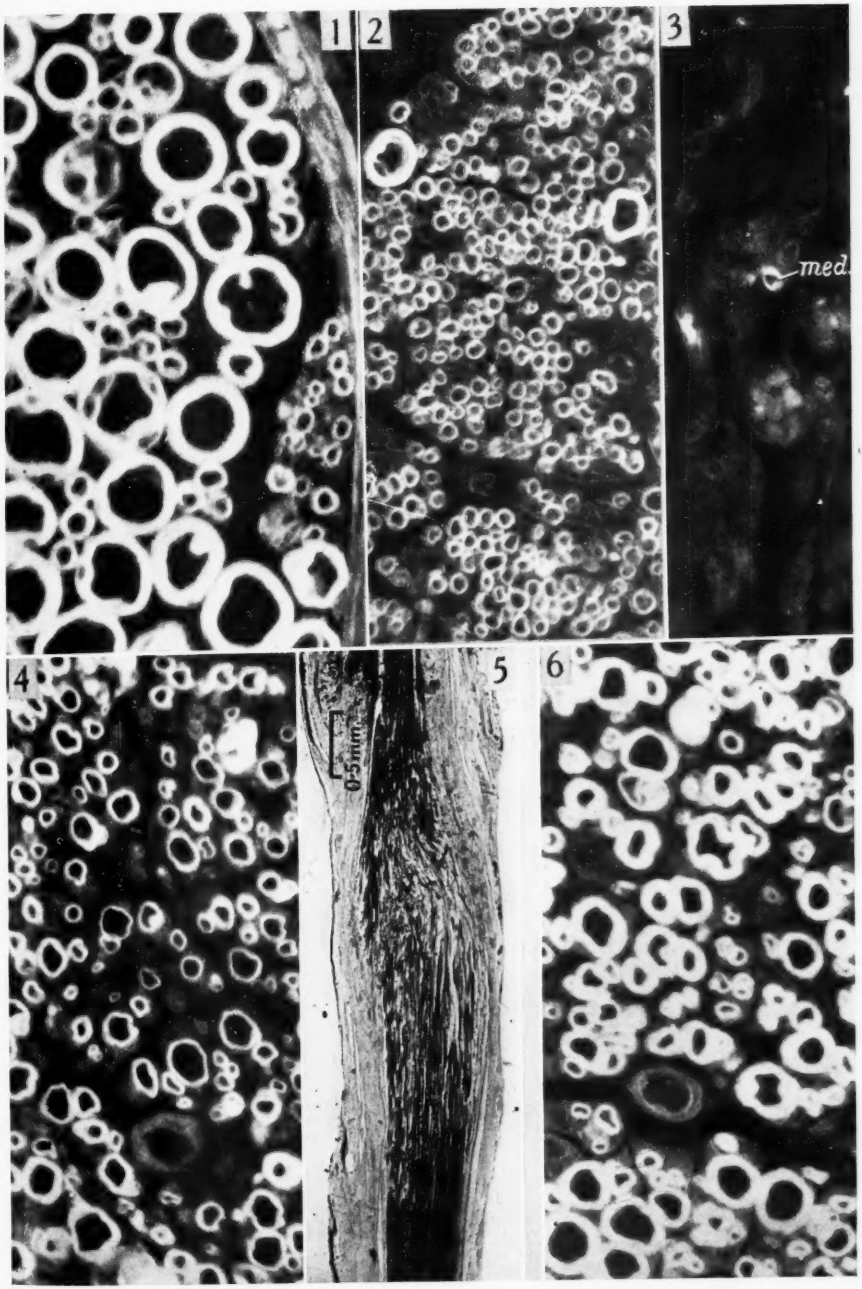
Fig. 1. Portion of normal somatic nerve (iliohypogastric, D13) of rabbit, showing large and small fibres (999*c*). This and all the similar figures are of material fixed in Flemming's fluid and stained with Weigert. The photographs are negatives, made by direct projection on to bromide paper, all at a magnification of ×750.

Fig. 2. Normal great splanchnic, mostly small fibres (999*b*).

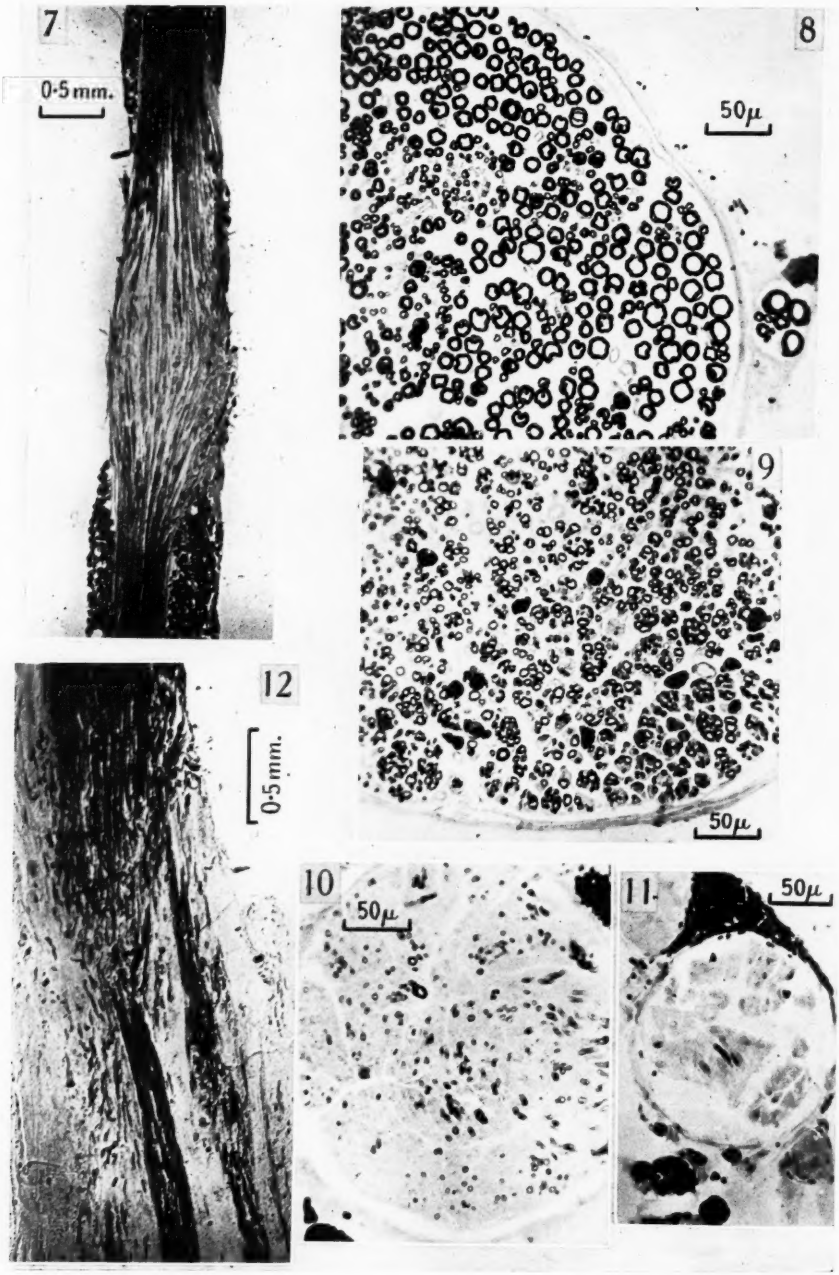
Fig. 3. Normal anterior mesenteric (732*a*). Only one very small medullated fibre (*med.*) is present.

Fig. 4. Somatic nerve 100 days after simple union with its own central stump (847*d*). Except where stated all figures after suture are of transverse sections 10 mm. below the union.

Fig. 5. Longitudinal section of simple somatic union after 100 days (847*d*). In this and all the figures of unions the central stump is above.



SIMPSON AND YOUNG—REGENERATION OF VISCERAL AND SOMATIC NERVES



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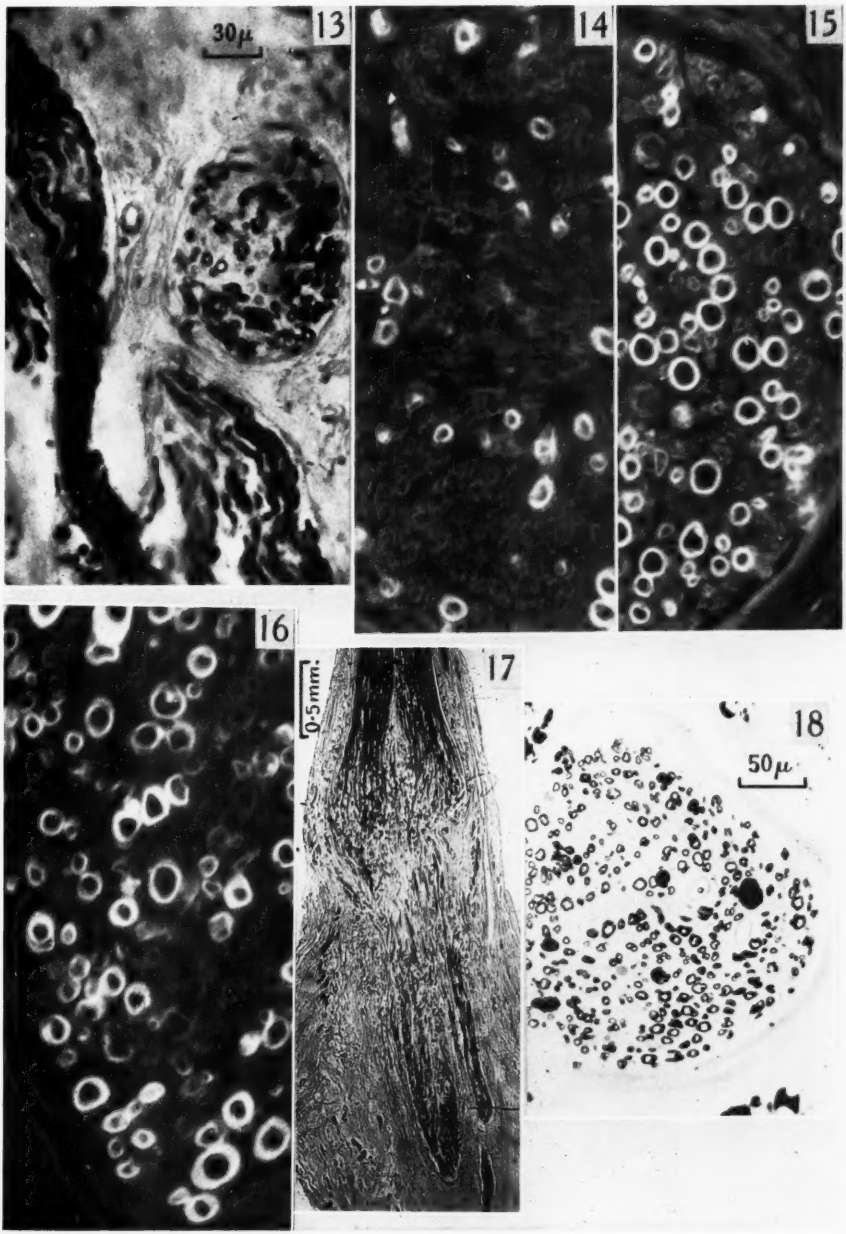
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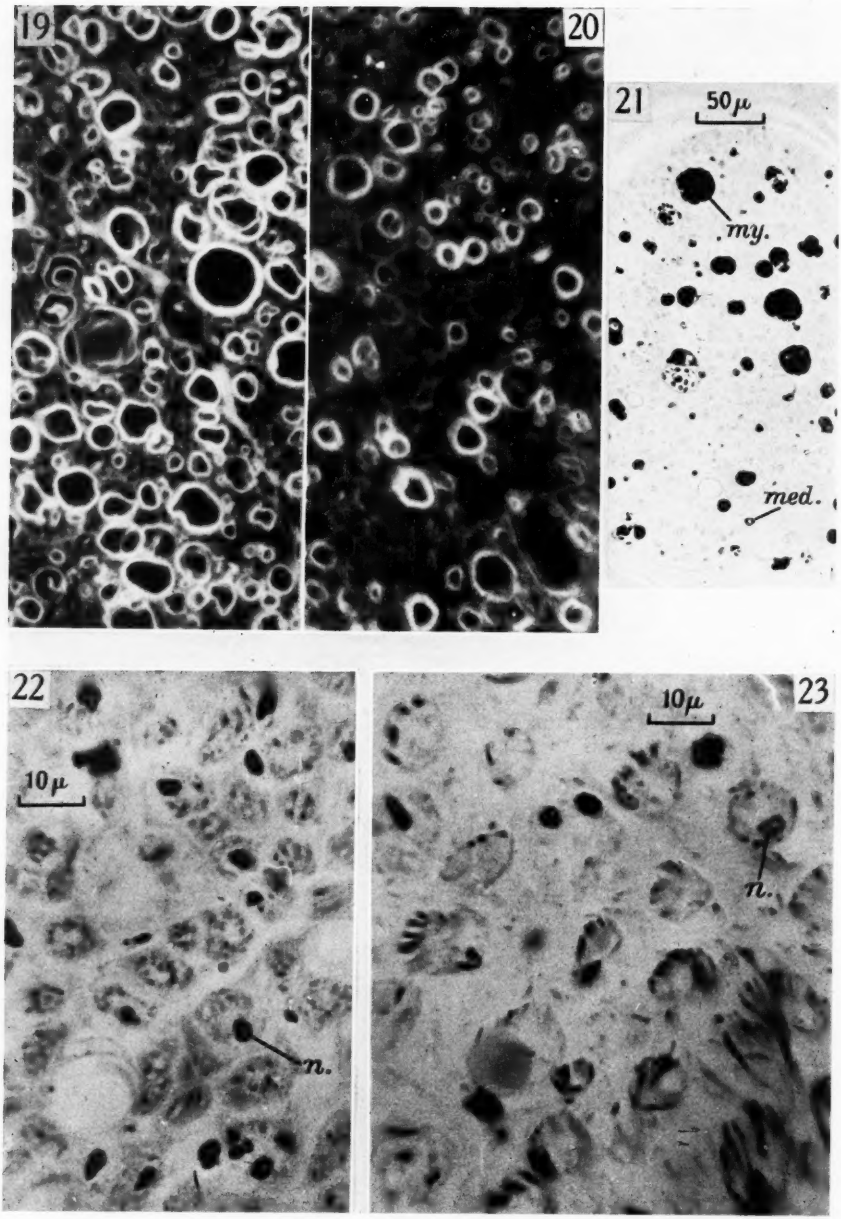

SIMPSON AND YOUNG—REGENERATION OF VISCERAL AND SOMATIC NERVES

Fig. 6. Somatic nerve 200 days after simple union with its own central stump (811*l*).

PLATE 2

Fig. 7. Longitudinal section of simple somatic union 200 days (811*l*).

Fig. 8. Central stump of somatic nerve 100 days after grafting to a piece of somatic nerve (842*a*).

Fig. 9. Portion of one somatic nerve grafted into another (100 days) (842*a*). Note very large number of fibres, often several in each tube. This is an unusual case in that there are more fibres in the peripheral than in the central stump.

Fig. 10. Splanchnic nerve 100 days after union with its own central stump (854*e*). Composition similar to that of normal nerve.

Fig. 11. Anterior mesenteric nerve 100 days after union with its own central stump (853*m*). Composition similar to that of normal nerve, containing only a few, small, medullated fibres.

Fig. 12. Low-power view of same slide as Pl. 3, fig. 13, to show union of a somatic nerve with the bundles of the anterior mesenteric, and medullated fibres in the latter (833*a*).

PLATE 3

Fig. 13. Anterior mesenteric innervated for 100 days by a somatic nerve (833*a*). Numerous small medullated fibres seen in transverse section and longitudinal section.

Fig. 14. Anterior mesenteric innervated for 100 days by a somatic nerve (735*b*). Many small medullated fibres present.

Fig. 15. Anterior mesenteric innervated for 200 days by a somatic nerve (811*b*). Fibres more numerous than after 100 days but not much larger.

Fig. 16. Splanchnic nerve innervated for 100 days by a somatic nerve (833*c*).

Fig. 17. Longitudinal section of union of somatic and splanchnic nerves (833*c*).

Fig. 18. Splanchnic nerve innervated for 100 days by a somatic nerve (853*c*).

PLATE 4

Fig. 19. Splanchnic nerve innervated for 200 days by a somatic nerve (811*f*).

Fig. 20. Somatic nerve innervated for 100 days by a splanchnic (959*f*). The fibres are larger than those of the splanchnic, but the possibility of invasion from the muscles must be borne in mind.

Fig. 21. Somatic nerve innervated for 100 days by anterior mesenteric (834*a*). At most a very few possible medullated fibres (*med.*). Numerous myelin remains (*my.*).

Fig. 22. Somatic nerve innervated for 100 days by anterior mesenteric (834*d*). Bodian stain showing unmedullated nerve fibres in the Schwann tubes. *n*, Schwann cell nucleus.

Fig. 23. Somatic nerve innervated for 200 days by anterior mesenteric (958*a*). Bodian stain. The unmedullated fibres are perhaps slightly larger than 100 days after union.

THE CELL CONTENT OF SYNOVIAL FLUID

By D. V. DAVIES, *Anatomy School, Cambridge*

Investigations on the cell content and cytology of normal synovial fluid are still scanty. The results so far published are summarized in Table 1, which shows clearly the inadequacy of our knowledge on the subject, not only in animals but also in man. Of the latter only the figures given by Coggeshall and co-workers seem worthy of attention. It is doubtful whether these figures indicate the true range found in normal knee joints since the fluids were obtained from patients of 32–80 years of age, and it is possible that some of the joints may have shown degenerative changes. These patients suffered from chronic diseases and led a life of relative, if not almost complete, inactivity for some time before death, so that their joints had not been subject to the normal wear and tear processes. It would be reasonable to expect that the wear and tear of normal movement would be reflected in the cell content of the fluid. This may account to some extent for the very low average value given by these authors. All the joints examined were in the limbs; there are no data on joints of the trunk. The average cell count in the knee-joint fluid of dogs recorded by Warren, Bennett & Bauer is relatively high. The age of the dogs and the condition of the joints are not stated. There are two possible explanations of this high figure. Either one is dealing with a joint subject to great functional activity or with a true species difference. Existing information is insufficient to determine the influence of these two factors.

Table 1. *Résumé of the literature on the content of nucleated cells in normal synovial fluid*

Author	Date	Animal	Joint	No. of observations	No. of nucleated cells			Remarks
					Max.	Min.	Average	
Bauer, Bennett, Marble & Claflin	1930	Cattle	Astragalo-tibial	63	252	55	112	—
Do.	1930	Cattle	Carpo-metacarpal	15	492	95	222	Not considered normal by authors due to supposed constant degenerative changes in this joint in cattle over 2 years of age
Coggeshall, Warren & Bauer	1940	Human	Knee	29	180	13	63	—
Forkner	1930	? Human	?	?	—	—	±50	—
Key	1928	Rabbit	Shoulder	? Large series	375	80	Majority between 175 and 225	Author states that similar counts were obtained from joints of children and other laboratory animals
Kling	1931	Human	?	18	50	0	?	—
Labor & Balogh	1919	Human	?	?	20	10	?	—
Policard	1936	?	?	?	250–300	75	?	—
Ropes, Bennett, & Bauer	1939	Cattle	Astragalo-tibial	?	250	65	131	—
Do.	1939	Human	?	29	?	?	63	Probably referring to series by Coggeshall *et al.* (1940)
Warren, Bennett & Bauer	1935	Cattle	Astragalo-tibial	25	575	55	181·8	Admit an error in the differential count in Bauer, Bennett, Marble & Claflin's series of 1930
Do.	1935	Cattle	Carpo-metacarpal	12	555	100	213·3	Young beef cattle
Do.	1935	Dog	Knee	14	1450	327	963·8	—
Do.	1935	Rabbit	Knee	?	330	140	242·5	—

MATERIAL AND METHODS

Synovial fluid has been examined from a large series of cattle and sheep and from a small number of horses. The fluids were obtained by aspiration with needle and syringe within half an hour or less of the animal being slaughtered. The joints examined were the atlanto-occipital, atlanto-epistrophial, temporo-mandibular and costo-vertebral in the trunk; the knee, astragalo-tibial, elbow and radio-carpal in the limbs. Of the costo-vertebral joints only those between the heads of the ribs and the bodies of the vertebrae were examined. The

approaches to the atlanto-occipital joint and the joints of the limbs have been described in a previous paper (1944). The atlanto-epistrophial joint was approached from the ventral side after removal of the head. In cattle and sheep the left and right atlanto-epistrophial joints are continuous with the joint between the odontoid process and the anterior arch of the atlas. The temporo-mandibular joint was approached from its postero-lateral aspect, the needle being passed upwards, forwards and inwards into the joint socket. The costo-vertebral joints could only be approached from within the thoracic cavity after the carcase had been eviscerated. A small separation of the slips of the longus colli muscle was necessary before the first and second of these joints could be palpated.

The cell counts were made on a Fuchs-Rosenthal cerebrospinal fluid counting chamber in the case of the appendicular joints, and on a Hawksley blood counting chamber, using the $\frac{1}{250}$ sq.mm. squares in the case of the axial joints. The cells in 1 cu.mm. were counted (except in those cases where the count per cu.mm. totalled over 1000 when the cells in $\frac{1}{2}$ cu.mm. were counted). No dilution of the fluid was made before counting as the cell count is low and the fluid of varying viscosity. To facilitate counting, the nucleated cells were stained by smearing a longitudinal band of 1% alcoholic cresyl violet on the coverslip, allowing it to dry, and then inverting it on a drop of synovial fluid placed in the well of the counting chamber. Adequate staining was obtained within a few minutes.

RESULTS

Table 3 gives details of the cell counts in the knee joints of cattle. The atlanto-occipital joint was investigated in two different series of sheep; these results are recorded in Tables 6 and 7. The first series consisted of fifteen pregnant ewes of about seven years of age (Table 6), the second of seventeen 4-month-old lambs (Table 7). In addition the cells in the atlanto-occipital joints of a single 1-year-old lamb were examined (see footnote to Table 5). The very high cell counts recorded here are worthy of note. These two series of sheep were all slaughtered at the termination of a nutrition experiment and details of the feeding are recorded in Tables 6 and 7.

Except for the figures shown in Table 6, the red cell count of the synovial fluid is not recorded. The red cells were counted in all cases and fluids containing over 1000 red cells per cu.mm. were discarded as too heavily contaminated with blood or otherwise abnormal. The presence of small numbers of red cells in normal synovial fluid must be regarded as due to accidental contamination during aspiration. More than half of all fluids examined contained no red cells (see Table 6), and as a general rule the average number of red cells per cu.mm. was considerably lower in those joints which contained large volumes of synovial fluid, such as the hock in cattle, than in joints with a small volume of fluid. This suggests that there was a greater dilution of the blood accidentally carried into the fluid during puncture. In the case of the bilateral joints there was little correspondence between the number of red cells in the joints on the right and left sides and no constant relation existed between the number of nucleated cells and the red cell content.

Gross contamination with blood was encountered in a small number of cases. The highest proportion of such cases was in the series of 4-month-old lambs (Table 7) when five specimens out of a possible total of thirty-eight had to be discarded for this reason. These were probably cases where haemorrhage had occurred into the joint at the time of slaughter, the red cells being still intact and not haemolysed. Small breaches in the synovial membrane, where the haemorrhage had occurred, were found in these cases, although no major tear of the capsule was discovered. The only restraint exercised on these animals was that required to keep them under control, all handling being done with the utmost gentleness. This illustrates the ease with which injury and haemorrhage can occur at the synovial membrane, and it is not surprising when the extreme vascularity and the superficial nature of the capillary net at the surface of the synovial membrane are considered. It appears that young animals are more susceptible to this type of injury than the older ones, possibly due to difficulty in controlling them, although the greater vascularity of the synovial membrane at this age must not be excluded as an additional factor. Gross contamination with blood was unusual in the old horses examined (Table 8).

Differential counts of the nucleated cells in synovial fluid were not made, although it was clear that most, if not all, the fluids examined showed a preponderance of monocytes and clasmatocytes over lymphocytes, while polymorphonuclear leucocytes formed only a very small percentage of the total. All fluids containing more than an occasional polymorphonuclear cell were discarded as abnormal. A mild form of apparently infective arthritis, in which the fluid was turbid from its high cell content, was not uncommon in the hind-limb joints of the 1-year-old lambs examined. These showed fluids with a marked preponderance of polymorphonuclear cells. These cases have not been recorded. At a later date it is hoped to present a detailed study of the differential nucleated cell count, particularly with regard to any variations from joint to joint and in different functional states of the joint.

In cattle all the axial joints are characterized by the highly viscous nature of their fluid, less marked perhaps in the temporo-mandibular joint than in those related to the atlas. These joint fluids are also

characterized by their marked yellow colour, the pigment present being carotinoid in nature. The fluid from the first costo-vertebral joint (i.e. that associated with the first rib) was frequently much less viscous than that in the remainder of the series. Furthermore, the costo-vertebral joint fluids were as a rule much less deeply pigmented than the fluids from the joints related to the atlas or the temporo-mandibular joint. Fluids from all the equine joints examined were invariably deeply pigmented, while none of them approached the fluids from bovine trunk joints in their viscosity. In general they were much more uniform in character than cattle fluids. In sheep, on the other hand, the fluids from both limbs and trunk tended to be uniformly highly viscous and colourless. Occasional samples of synovial fluid were noticeably yellow despite the absence of any red cells. The nucleated cell content of these pigmented fluids did not suggest that they were abnormal.

There was generally a close correspondence in the volumes of fluid between the homologous joints on the right and left sides of the body. In a few cases there was a marked discrepancy, as for example in the 4-month-old lambs 41, 47 and 50 (Table 7). In lamb 41 this lack of correspondence in regard to volume also characterized the cell content. Lamb 47, however, showed no parallel discrepancy in nucleated cell content, those in the right and left atlanto-occipital joints being similar. In lamb 50 the right atlanto-occipital joint yielded insufficient fluid for a satisfactory cell count, while the left joint yielded about 0·4 c.c. of fluid. The completeness of the aspiration was verified in all these cases by subsequent direct inspection. There seemed little to account for these discrepancies. No evidence of recent trauma could be discovered, but a more remote injury, although unlikely, could not be excluded. In macroscopic inspection the articular structures appeared normal.

In regard to the nucleated cell content, Tables 6 and 7 serve to illustrate the close agreement which generally existed between corresponding joints on the right and left sides of the same animal. This relation existed for all bilateral joints examined.

In the cattle examined (Tables 2 and 3) it will be seen that the average figure of 194 cells per cu.mm. for the astragalo-tibial joint is in close agreement with that of 181·8 cells per cu.mm. given by Warren *et al.* (1935). Both these figures, however, differ appreciably from that of 112 cells per cu.mm. recorded by Bauer, Bennett, Marble & Claflin (1930) for fluid from this joint. It is difficult to account for this anomaly. There are several possible explanations, such as the small number of observations in the present investigation compared with the number recorded by Bauer *et al.* (1930); lack of correspondence in the ages of the animals examined in the two groups; or a different functional state of the joints examined in regard to movement and exercise prior to aspiration. The possibility of failure to recognize all the very small lymphocytic cells which are prevalent in this joint must not be overlooked.

In both cattle and sheep it will be noticed that there is a significant difference between the nucleated cell content of fluids from appendicular joints and those from the joints of the head and neck. There is a similarity in the corresponding fluids as regards cell content in these two groups of animals. In the case of the atlanto-occipital joint of sheep, series 1 (Tables 5 and 6) was examined first together with the 1-year-old lamb recorded in the footnote to Table 5. Here the relatively low cell content of fluids from the 7-year-old sheep attracted attention, together with the very high content of the specimens from the 1-year-old lamb. The possibility of age and state of nutrition affecting the count was considered. Table 6 shows that there is no close correspondence between the absolute weight of the animals and the cell count, but, with the exception of E18 and E21, all animals showing the lower cell counts had been grossly underfed during the 4-month period immediately preceding slaughter. It was difficult, however, to accept any direct relation between nutrition and cell content in joint fluids. The cell content is probably only indirectly related to the feeding, in so far as starvation leads in due course to increasing weakness and a disinclination to undertake physical exercise. In a similar manner nutrition on a high plane and fatness may have as a consequence a lessening of the activity of the animal. This may account for the position of E21 in Table 6, this ewe being the heaviest of the series and bearing only one lamb. The unexpectedly high cell content of E18 may be explained by two facts, first the short period of 2 months of underfeeding and secondly the bearing of a single lamb only, resulting in a much smaller load imposed on the maternal organism. Apart from these two ewes, the remaining sheep shown in Table 6 bore twins.

Investigations on the influence of age on the cell content of synovial fluid were carried out in an attempt to account for the difference between the atlanto-occipital joint fluids of cattle and sheep and for the high cell content of the 1-year-old lamb (Table 4). The fluids from the atlanto-occipital joints of a series of 4-month-old lambs (Table 5, series II and Table 7) and from the knee joints of a series of calves under 3 months of age (Table 3) were examined. It is clear that the results recorded in Table 7 tend to confirm the impressions previously gained in examination of the pregnant ewes. All lambs on a low plane of nutrition for the 8 weeks prior to slaughter yielded fluid with relatively low cell content, whilst the very heavy lambs (nos. 45 and 51) gave values intermediate between the well

Table 2. *Nucleated cell content of synovial fluid per cu.mm. in cattle*

Joint	No. of observations	Maximum	Minimum	Average	Remarks
Atlanto-occipital	22	1208	542	783·9	All from cattle of 1–4 years of age
Atlanto-epistrophial	21	1604	549	888·4	All from cattle of 1–4 years of age
Temporo-mandibular	22	1756	800	1337·5	All from cattle of 1–4 years of age
Knee	41	483	86	246·6	Analysed in greater detail in Table 3
Astragalo-tibial	21	316	50	194·2	All from cattle of 1–4 years of age
Elbow	15	500	53	197·3	All from cattle of 1–4 years of age
Radiocarpal	20	530	130	246·2	All from cattle of 1–4 years of age

Table 3. *Nucelated cell content of synovial fluid from cattle knee joints*

Description	No. of observations	No. of nucleated cells per cu.mm.		
		Maximum	Minimum	Average
Calves 0–3 months old	12	483	160	263·8
Cattle 1–2 years old	9	346	214	277·5
Cattle 3–4 years old	20	470	86	222·4
Total	41	483	86	246·6

Table 4. *Nucleated cell content of synovial fluid from costo-vertebral joints in cattle*

No. of animal	Side	Cell content per cu.mm.									
		Joint 1	Joint 2	Joint 3	Joint 4	Joint 5	Joint 6	Joint 7	Joint 8	Joint 9	Joint 10
1	L	—	—	668	—	—	—	—	—	—	—
2	L	—	374	—	—	—	—	—	—	653	—
3	R	—	—	—	624	—	608	—	—	—	—
4	R	—	—	—	292	—	330	—	—	—	—
5	R	622	—	—	—	—	—	—	—	—	—
6	L	904	—	—	1012	—	—	—	—	—	—
7	L	734	—	896	—	—	—	—	—	—	—
7	R	—	822	—	—	632	—	—	—	—	—
8	L	474	—	—	—	—	—	—	—	—	—
8	R	472	726	—	—	—	—	—	—	—	—
9	L	328	—	—	720	—	—	—	—	968	—
10	L	750	736	—	678	638	794	858	968	930	—
11	L	500	—	478	712	642	608	712	—	—	—
11	R	562	412	596	—	—	—	—	—	—	—
12	L	162	646	690	650	—	656	—	—	—	—
13	R	248	458	532	692	664	588	512	—	—	616
14	R	502	658	722	—	—	—	—	—	—	—
15	R	258	—	—	—	—	—	—	—	—	—
16	L	472	496	608	456	—	—	—	—	—	—
16	R	164	410	406	—	—	—	—	—	—	—
17	R	306	756	782	—	—	—	—	—	—	—
18	L	130	—	442	—	—	—	—	—	—	—
19	L	330	—	446	622	—	—	—	—	—	—
20	L	378	—	—	—	—	—	—	—	—	—
21	L	446	350	528	566	608	698	—	—	—	—
21	R	—	438	—	—	—	—	—	—	—	—
22	R	—	686	—	610	—	—	—	—	—	—
23	L	402	—	—	—	—	—	—	—	—	—
24	R	734	1048	860	902	852	—	—	—	—	—
24	L	730	1018	856	—	—	920	—	—	—	—
25	R	658	812	852	854	632	—	—	—	—	—
26	R	202	408	674	614	—	—	—	—	—	—
27	R	636	996	674	—	—	—	—	—	—	—
28	L	—	646	722	—	—	—	—	—	—	—
29	L	226	948	814	804	896	1024	1140	—	—	—
29	R	202	946	852	892	838	1078	1102	1128	884	—
Total no. of observations		28	22	21	17	9	10	5	2	4	1
Average		450·4	692·3	677·3	688·3	711·3	730·4	864·8	1048	858·8	616

Table 5. *Nucleated cell content of synovial fluid in sheep per cu.mm.*

Joint	No. of observations	Maximum	Minimum	Average	Remarks
Atlanto-occipital* (series I)	18	1292	228	611·7	All pregnant ewes of about 7 years of age
Atlanto-occipital† (series II)	31	1974	602	1110·0	All from lambs of 4 months
Atlanto-occipital‡ (series I and II)	49	1974	228	926·9	—
Atlanto-epistrophial	14	1960	728	1060·7	All 1-year-old lambs
Temporo-mandibular	13	1478	347	993·5	
Knee	16	519	113	254·7	
Astragalo-tibial	24	405	81	207·3	
Elbow	19	411	73	200·1	
Radio-carpal	15	298	73	157·5	

* See also Table 6.
† See also Table 7.
‡ Counts from this joint in a 1-year-old lamb have not been included. In this case the right and left atlanto-occipital joints gave counts of 2606 and 2126 respectively.

Table 6. *Nucleated cell content of the atlanto-occipital joint fluid in 7-year-old pregnant ewes (Table 5, series I)*

No.	Nucleated cells per cu.mm. Right	Left	Average	Red blood cells per cu.mm. Right	Left	Average	Plane of nutrition* in months	Live weight at slaughter in lb.	No. of lambs	Remarks
E 19	1292	1178	1235	0	0	0	Low 4, then high 2	175	2	At term
E 20	760	844	802	72	130	101	Low 2, then high 4	186	2	At term
E 18	660	714	687	24	2	13	High 4, then low 2	133	1	At term
E 27	680	524	602	16	36	26	High 6	148	2	Aborted at 91 days
E 23	610	582	596	342	228	285	High 2, then low 4	148	2	At term
E 21	600	580	590	0	0	0	High 6	200	1	At term
E 17	374	458	416	0	166	83	High 2, then low 4	148	2	Aborted 48 hr. before slaughter
E 22	338	324	331	0	0	0	Low 4	115	2	At term
E 28	228	264	246	0	0	0	Low 4	113	2	Aborted on day of slaughter

* All above were on a maintenance diet of 4½ lb. hay per day for 2 months before nutrition experiment commenced, thereafter low plane was 1½ lb. per day and high plane was 9 lb. per day.

Table 7. *Nucleated cell content of synovial fluid from the atlanto-occipital joints of 4-month-old lambs (Table 5, series II)*

No. of lamb	Nucleated cells per cu.mm. Right	Left	Average	Nutrition plane* 0–8 weeks	8–16 weeks	Live weight at slaughter in lb.
41	1588	1974	1781	High	High	69·6
27	1656	—	1656	Low	High	79·6
28	1548	1534	1541	Low	High	64·1
50	—	1474	1474	High	High	73·7
37	1324	1582	1453	Low	High	61·3
45	1408	1222	1315	High	High	97·4
34	1264	1134	1199	Low	Low	56·4
51	1306	1084	1195	High	High	82·4
39	—	1184	1184	Low	Low	45·8
32	974	1046	1010	High	High	68·1
43	962	900	931	High	Low	64·6
48	896	862	879	Low	Low	49·9
36	866	712	789	Low	Low	54·0
42	810	752	781	High	Low	66·0
38	956	602	779	Low	Low	33·0
46	716	734	725	High	Low	72·5
47	698	644	671	High	Low	64·6

* Lambs suckling high yielding ewes during first 8 weeks of life are designated high. From 8 to 16 weeks lambs designated high received a liberal supply of supplementary food whilst those designated low had their supplementary feeding severely restricted.

and poorly fed animals. This, however, does not explain the difference which existed between these two series of sheep and a marked reduction in cell content with age is suggested. In the cattle series (Table 3) a parallel reduction of cells with age does not occur; the differences shown between the various age groups could be explained equally well by the small number of observations made. Any age change which may still occur here cannot be of the same magnitude as that seen in the atlanto-occipital joint of sheep. With regard to this possibility it is well to consider the differences obtaining between the young of these two species. The calf in the early months after birth prefers to spend the intervals between suckling or meals lying down in the yard or the field, whilst the young lamb spends much of the time in play and is generally much more active than its mother. In the natural state the lamb begins to ruminate at 4–5 months and the calf at about 9 months. Exercise and amount of movement must be considered as highly important factors in deciding cell content of synovial fluid, but species differences must not be eliminated. It is interesting to note that lambs 38 and 39 were twins and that the latter was the heavier and had the higher cell content in its synovial fluid.

Differences between the various joints in the one animal might be explained, in part at least, by variations in the amount and range of their movement, that is, variations in wear and tear within the joint. The relatively high cell count in the temporo-mandibular joint of the ruminants examined supports this contention. The axial joints in both cattle and sheep with their highly cellular and highly viscous fluids are also characterized by their freedom from degenerative processes, since contact is prevented between the surfaces during any phase of movement or while at rest, as obtaining in 'perfect' lubrication. In the axial joints this is combined with the transmission of a relatively small load through the joint as compared with that transmitted through the appendicular joints. On the other hand, the highly viscous nature of the synovial fluid in the former may hinder removal of debris from the joint, compensation for this being provided in the form of a highly cellular fluid rich in phagocytes. In this respect it is interesting to note that these cellular fluids appear to contain a greater proportion of phagocytic cells of the clasmatocyte or monocyte series than do the appendicular joint fluids, where the lymphocytes are much more numerous. This does not account for the fact that, though the appendicular joint fluids in sheep are similar in their cell content to those in cattle, yet as a rule they are much more viscous. As regards viscosity they are much more closely allied to those in the appendicular joints of dog. Warren *et al.* have already reported a high nucleated cell count in fluids from the knee joints of dogs, and this observation has been confirmed for the knee joint and other appendicular joints. This perhaps argues in favour of a true species difference, not determined solely by the activity of the species in question; that is, a difference in cell content would exist were the joints immobilized.

Table 8. *Nucleated cell content of synovial fluid in horses per cu.mm.*

Joint	No. of observations	Maximum	Minimum	Average	Remarks
Atlanto-occipital	5	1162	358	594	—
Atlanto-epistrophial	10	678	346	534	—
Temporo-mandibular	14	2350	412	983*	Majority of counts were between 650 and 850
Knee	16	1638†	390	671	All specimens except three gave values between 400 and 1000
Astragalo-tibial	18	368	72	192	—
Elbow	12	336	107	207	—
Radio-carpal	17	453	50	234	—

* If three specimens of 2350, 2016 and 1588 cells per cu.mm. are excluded, the average value becomes 710.
† The opposite knee joints in one of these animals gave unusually high cell counts of 1638 and 1266 respectively.

In the horses examined (Table 8) the relatively low cell counts for the atlanto-occipital and atlanto-epistrophial joints should be noted. Counts for the former bear a similarity to those recorded for the corresponding joint in 7-year-old ewes (Table 5, series I). Further, all these counts were from aged animals. The other feature worthy of note in Table 8 is the high average count in fluid from the temporo-mandibular joint. Although this does not approach the figure for the same joint in cattle, it approximates closely to that found in sheep. Counts from other non-ruminating animals may help to explain this difference.

Table 4 gives the data for the costo-vertebral joints in cattle. Only a single specimen from the tenth costo-vertebral joint was examined and none from the joints caudal to this. The reason for this lies in the volume of fluid available on aspiration. This was maximal at about 0·3–0·4 cu.mm. in the first costo-vertebral joint and decreased rapidly as

one passed from joint to joint in the caudal direction. Differences in the viscosity along this series have been mentioned already. The relatively immobile first costo-vertebral joint shows a much lower cell content per unit volume than its successors. As far as the eighth joint there is a tendency to a progressive increase in cell content but further figures are required before any categorical statement to this effect can be made. The lower average counts of the costo-vertebral series as compared with those of the head and neck need explanation. Despite constant movement the wear and tear of the joint surface in the former may be considerably minimized on account of the small range of movement and the nicety of its control both as regards regularity and smoothness, particularly during reversal of movement. The latter factor would certainly tend to conserve the integrity of the joint surfaces and diminish any tendency to excessive wear within the joints. In all animals degenerative changes in the joints involved in thoracic respiratory movements are extremely rare.

CONCLUSIONS

From the foregoing analysis it appears that not only do the various joints in the same animal differ in their nucleated cell content, but that the latter may vary quite markedly in homologous joints of different species. As already suggested in a previous paper (1944), similar differences exist in respect of other characters of synovial fluid, such as viscosity and pigmentation. The variation in the cell content is related, in part at least, to the functional activity of the joints concerned and in particular to the wear and tear occurring within the joint.

As a rule the joints of the trunk show a high nucleated cell content compared with those of the limbs. These relatively cellular fluids tend to be highly viscous. The high viscosity of synovial fluids may hinder removal from the joint of small particulate matter resulting from wear and tear. An increase in the phagocytes within the joint may compensate for such a deficiency. It is worthy of note that these highly viscous fluids show a marked predominance of the phagocytic type of cell of the clasmatocyte or monocyte series as compared with the less viscous and relatively acellular fluids most of which frequently show higher proportions of lymphocytes.

An interesting feature of the joints containing the more cellular fluids is their relative freedom from degenerative changes. The temporo-mandibular, atlanto-occipital, atlanto-epistrophial and costo-vertebral joints are all relatively free from disease and degenerative changes in both animals and man. This freedom from pathological and age changes is associated with highly viscous fluids of high cell content.

Little is known of the cell content of human synovial fluid. The figures so far published do not allow any deductions as to the effects of age or activity. Nothing is known of any variations which may occur from joint to joint in man. Species differences in the cell content, unrelated to the amount of movement, probably occur, but it is often difficult to dissociate these from the species differences resulting from variations in the amount and range of mobility within the joint. This problem could only be solved by comparing the different species following an adequate immobilization of the joint in question.

SUMMARY

1. Counts of nucleated cells in both appendicular and axial joints of cattle, sheep and horses are recorded.

2. Significant variations are shown to exist between axial and appendicular joints, particularly in cattle and sheep.

3. The differences in nucleated cell content from joint to joint in the different species are related to the movement at the joint as regards frequency, extent and smoothness of control.

4. High nucleated cell counts generally occur in highly viscous fluids in joints characterized by freedom from disease and degenerative changes.

5. Red blood cells do not normally appear in synovial fluid. Their presence in small numbers is generally attributable to contamination during aspiration.

6. Haemorrhages occur comparatively easily into joint cavities, a fact which is to be correlated with the vascularity at the synovial surface. The younger animal is the more susceptible to this type of injury.

7. Clasmatocytes and monocytes are the predominant cells in synovial fluid. Polymorphonuclear cells are few in number; if more than an occasional polymorphonuclear cell is found, the fluid must be regarded as abnormal. It is probable that the differential cell count varies from joint to joint and with the functional state of a particular joint.

8. It is probable that species variations, unrelated to movement, also exist. Confirmation of this involves comparison of the joint fluids after adequate immobilization.

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THE EFFECT OF INTERFERENCE WITH THE BLOOD SUPPLY ON THE REGENERATION OF PERIPHERAL NERVES

By P. BACSICH and G. M. WYBURN, *Department of Anatomy, University of Glasgow*

INTRODUCTION

From recent experiments on the crushed sciatic nerve of the rabbit (Bacsich & Wyburn, 1945) we found that after destroying the regional nutrient arteries the circulation of a peripheral nerve remains normal. If, in addition, the epineural longitudinal anastomoses are also destroyed there is some early evidence of ischaemia, but the circulation is restored to normal within 14 days. As then stated these results required to be supplemented by a comparison of the rates of regeneration of injured nerves under the same experimental conditions.

In this work, after similar treatment of the blood vessels, we investigated histologically the course of nerve degeneration and regeneration in the first 30 days. Any assessment of the rate of regeneration of a nerve is ultimately a measure of the speed of functional recovery, and it might be objected that the method of histological observation neglects the later processes of maturation (Gutmann, Guttmann, Medawar & Young, 1942; Young, 1942)—the prelude to the return of function. It seems reasonable, however, to assume that any reaction to vascular derangement would be immediate rather than remote, and if the later phases of regeneration are affected by mobilization of the nerve and severance of the collateral nutrient vessels, there will be evidence of retarded and abnormal regeneration during the earlier stages. Especially should this be so where the longitudinal anastomoses are interrupted with consequent ischaemia.

Our task is therefore to detect the presence or confirm the absence of any significant change in the early regeneration picture after interference with the blood supply.

MATERIAL AND METHODS

The experiments were carried out on the sciatic nerve of the rabbit. The details of the operation for exposure of the nerve have been described elsewhere (Bacsich & Wyburn, 1945). In the first series of experiments the right and left nerves of each animal were exposed. On the right side the nerve was approached through a 1 in. incision, gripped and severely crushed with Spencer-Wells artery forceps. There was no traction on the nerve or disturbance of its position. After a much larger incision and separation, but not cutting, of the flexors and extensors, the left nerve was similarly crushed. It was then freely mobilized from the sciatic notch to and beyond the point of terminal branching and all the collateral nutrient arteries were cut, i.e. the gluteal, femoral, and saphenous groups. On each side the lesion was made about the level of the junction of the upper and middle thirds of the thigh. Previously we had trouble with post-operative ulceration in our animals. In these experiments only a few animals developed small trophic ulcers. This we ascribe, in part, to improved post-operative treatment, but an undoubted preventative was the greater degree of immobilization following the double operation. In the second series the right nerve was crushed. The left nerve was crushed, mobilized, the collateral nutrient arteries cut, and in addition all the visible epineural longitudinal vessels above and below the crush were destroyed by scraping the surface with a Graefe cataract knife.

The animals were killed at 5-day intervals ranging from 5 to 30 days after operation and the affected portions of the nerves were carefully removed, including in the later stages considerable stretches of the main branches.

The fixative used was alcohol-acetic acid-formalin (Hofker's Fixative) which gives the necessary freedom for the different staining methods to be used on sections of the same material. Where a long stretch of nerve was removed it was cut into short portions of about 3 cm. All nerves were embedded in celloidin-paraffin.

A few cross-sections of 20, 10 and 5 μ were cut from the peripheral and central ends of the blocks which were then sectioned longitudinally at 10 μ with an odd 5 μ section marked for special study. The 10 μ longitudinal sections were mounted in two series—odd and even numbers.

Staining

(1) One longitudinal series plus some 10 μ cross-sections were stained with haemalum and eosin. These proved useful beyond our expectation not only for a comparison of the sections of the two sides but in the study of many of the finer structural details.

(2) The second longitudinal series with some 5 μ longitudinal and cross-sections were stained by a modified Protargol method (Bacsich, 1940) which

gives consistently good impregnation after one hour.*

(3) 5 μ longitudinal and transverse sections were treated with the Mallory triple staining method using MacConaill's mordant to ensure maximum colour range. Other similar sections were treated with Walter's modification of Pasini's triple stain. Both these stains give good results after silver impregnation.

(4) Some 10 μ longitudinal and 20 μ cross-sections were stained by a modified Weigert method (Bacsich & Stibbe, 1937).

The length of the advancing axons in the peripheral segments of the two sides was determined by independent observation and allowance made for the appropriate number of transverse sections cut from the peripheral ends.

As a further index of the relative progress of regeneration in the respective nerves a count was made of the number of axons per millimetre breadth of longitudinal section at a point 2 mm. below the centre of the crushed region. This was done in four sections of each nerve and the mean expresses an axon density for that nerve. Ideally, the axon density should denote the number of nerve fibres per unit of volume, i.e. in a cross-section. Our method was dictated by the necessity to measure the length and density of axons in the same nerve. The figures are of significance only as comparative data.

DESCRIPTION

First series of experiments

5 *days* (*animals* 1 *and* 3)

Peripheral segments. The picture is one of total nerve degeneration. Sections of both sides display all the varied shape and form of disintegration so meticulously described by Cajal (1928). Changes are uniform throughout the whole peripheral segment, except in the proximity of the crushed area, where there is evidence of a more rapid disintegration (Pl. 1, figs. 1, 2). On the whole, degeneration is slightly more advanced on the left side. On the right side there are considerable stretches of nerve fibres still in the early stages of degeneration—thickened, with irregular beading and varicosities—whereas on the left side stunted, coiled and vacuolated axonic fragments can be seen enclosed within the numerous digestive chambers (Pl. 1, figs. 1, 2). In accord with this more advanced phase is the increased silver impregnation of the non-neurofibrillar elements, which, as Weddell & Glees (1941) suggest, is due to 'diffusion through the tissues of the stainable substance of the axis cylinder'. In Weigert stained sections there is fragmentation and swelling of the myelin sheaths. Myelin degeneration is also more advanced on the left side.

There are no regenerated nerve fibres in either right or left sides. A very small number of thin fibres could be observed in various parts of the peripheral segments but in none of the sections could they be traced into the central segments. It was concluded that they were non-myelinated fibres which are more resistant to degeneration.

Central segments. It is difficult to determine the exact extent of the retrograde degeneration. Coiling and fragmentation of the axis cylinders, and fragmentation and swelling of the myelin sheaths can be followed for 7–8 mm. There is evidence of a greater damage on the left side and this difference is particularly well marked in animal No. 1.

Both sides already display signs of axonal regeneration—the 'metamorphic', or formative portions of axons terminate for the most part in neurofibrillar or brush-like expansions, and a few thin fibres can already be followed a short distance towards the crushed area.

10 *days* (*animals* 4 *and* 20)

Peripheral segments. 'Silver' preparations show that the regenerating axons have now bridged the crushed area and reached the peripheral segments. The mean advance of axons along the peripheral segment is 9·5 mm. on the right and 7·5 mm. on the left side. The mean difference of 2 mm. falls well within the normal range of variance and is not statistically significant. The mean fibre density is 26 on the right side and 24 on the left, the difference of 2 is again not statistically significant (Pl. 1, figs. 3, 4). There are large numbers of digestive chambers with mutilated and fragmented axis cylinders. All the non-myelinated fibres have now yielded to degeneration. These manifestations of degeneration are especially prominent on the left side of animal 20. In the 'Weigert' stained sections the positive staining can still be seen with the naked eye. Under the microscope fragmentation and distortion of the myelin sheaths is more advanced than it is in the 5-day group.

Central segments. Retrograde degeneration is more extensive on the left side and extends 7 mm. There are numerous fragmented axons and large terminal clubs, amongst which can be seen the thin, well stained regenerating fibres. On the right side there is little evidence of retrograde degeneration beyond 5 mm. 'Weigert' stained sections demonstrate the extent of retrograde degeneration and show the same slight difference between the two sides.

* Bayer's Protargol (pre-war) was used throughout.

Owing to a printer's error the following were omitted from the description of the method in the Proceedings:

After gold toning:

(1) Rinse sections rapidly in distilled water.

(2) Reduce for 5 min. in following solution:

Oxalic acid	2 g.
Formalin, conc.	1 c.c.
Distilled water	100 c.c.

15 *days* (*animals* 9, 14, 19, 22)

Peripheral segments (Pl. 1, figs. 5, 6). The mean axon advance in the peripheral segment is 22·3 mm. on the right side and 20 mm. on the left. The mean axon density is 31·5 on the right side and 35·7 on the left. By application of the '*t*' test neither the mean difference in length (2·3) nor in density (−4·2) of axons of the right and left sides is statistically significant. Degeneration in the peripheral segments of the two sides is about the same. Axon remnants are still a conspicuous feature, more so at a distance from the crushed area. There are a few short lengths of thickened axon but for the most part the remnants consist of twisted and coiled fragments and coarse granular debris in digestive chambers. Some distance from the crushed area the peripheral segments on both sides show dilated portions of old endoneurial tubes and more solid areas of Schwann tubes. The dilatations are mainly digestive chambers and a few due to incarcerated macrophages. The regenerated axons can be followed along both the solid and dilated parts of the tubes. Towards the crushed area the picture gradually changes. Masses of macrophages in single rows or in aggregations two or three cells deep replace the digestive chambers and are separated by Schwann bands and endoneurial elements.

The 'Weigert' sections show that there is still an appreciable amount of myelin residue.

Central segments. Signs of degeneration are less evident and are replaced by densely packed Schwann tubes with regenerating axons. The animals of this group show particularly numerous large club-shaped endings with some criss-crossing of fibres, Perroncito spindles, and other manifestations of frustration.

20 *days* (*animals* 2, 12, 21, 23)

Peripheral segments (Pl. 1, figs. 7, 8). The mean advance of regenerated axons on the right side is 36·5 and 36·3 on the left. The mean fibre density is 64 on each side. On both sides there are digestive chambers with degenerated axon and myelin fragments. These are more numerous in the distal parts of the peripheral segments where there are relatively fewer macrophages. Adjoining the crushed area are the macrophages in rows bordered by Schwann tubes with regenerating axons.

In the 'Weigert' sections a small number of nerve fibres, especially on the left side, have acquired a thin myelin sheath. Remnants of the old disintegrating myelin still persist.

Central segments. There is no difference between the two sides. The extent of the earlier degeneration is now indicated by the presence of Schwann tubes and macrophages. In animal 12 on the right side there are evidences of frustrated growth in the form of club-shaped swellings, criss-crossing of fibres and Perroncito spindles.

25 *days* (*animals* 7, 8, 13, 15, 18, 24)

Peripheral segments (Pl. 2, figs. 9, 10). The mean advance of the regenerated axons on the right side is 53·7 mm. and on the left side 49·5 mm., and the mean axon density is 66·5 on the right side and 70 on the left. By application of the '*t*' test neither the mean difference in length (+4·3) nor in density (−4·5) of the axons of the right and left sides is statistically significant. As in the 20-day group, in the neighbourhood of the crushed area the axons form irregularly spaced columns separated by rows of macrophages. Axon debris has been very largely cleared away from the proximal part of the peripheral segments, but more distally there yet remain short thickened fragments and coils of old axons. In this group it is difficult at times to distinguish with certainty between digestive chambers and a macrophage with a large, single vacuole in a dilated segment of a Schwann tube. At this stage the majority of the dilatations are due to macrophages.

In the 'Weigert' sections more nerve fibres have now acquired a delicate myelin sheath. The remains of old myelin can still be seen in the now infrequent digestive chambers, particularly on the left side.

Central segments. The two sides have the same structure and can be arbitrarily divided into two parts: (1) a peripheral part (i.e. adjoining the crushed area) which consists of Schwann tubes and rows of macrophages; (2) a central part where the structure approximates to that of normal nerve.

30 *days* (*animals* 5, 6, 10, 11, 17, 25)

Peripheral segments (Pl. 2, figs. 11, 12). The mean length of axon is 73·7 mm. on the right side and 69·5 mm. on the left, and the mean axon density is 85 on right side and 80·5 on the left. By application of the '*t*' test neither the mean difference in length (+4·2) nor in density (−4·5) of the axons of the right and left sides is statistically significant. The peripheral segments are not yet clear of axon remnants. The more distal parts in particular seem slow to sweep away the degenerative debris. Macrophages are still plentiful towards the crushed area.

In the 'Weigert' sections there are no longer traces of degenerating myelin. Many nerve fibres have now acquired a delicate myelin sheath.

Central segments. No difference between the two sides. Both sides show the same structure and division into two parts already discussed in the 25-day group.

Second series of experiments

Table 1 summarizes the results of the second series of experiments (Pl. 2, figs. 13–17). There is a persistent slightly greater growth length of the axon on the right side but the difference in the length of

the axons of the two sides at any one stage is well within the normal variance and therefore is not significant.

Animal 29 is of some interest. Both the length and density of axons is well below the mean for that period although the operation and post operative recovery were normal. Whatever the cause of the time lag the right and left sides are equally affected.

The structure and progressive histological changes of the nerves are the same on each side and are essentially similar to those already described for the respective periods in the first series of experiments. In the 10-, 15- and 20-day animals (nos. 30, 26 and 27) there is marked epineural thickening and connective tissue proliferation on the left side, particularly in the neighbourhood of the crushed area. This has diminished considerably in the 25- and 30-day animals (nos. 28 and 29).

Table 1

Animal no.	Days after operation	Length of axons in mm. Right side	Length of axons in mm. Left side	Number of axons per mm. Right side	Number of axons per mm. Left side
30	10	12	10	48	50
26	15	22½	18	60	68
27	20	41½	40	71	82
28	25	58	51	98	99
29	30	45½	47½	85	86

DISCUSSION

Cajal (1928) determined the growth rate of axons by histological examination. Young & Medawar (1940) and Gutmann *et al.* (1942) use the 'pinch reflex' method to ascertain the most advanced point of the growing axon tips, and the latter find a close correspondence between the figures so obtained and those from histological observation.

We believe that for our purposes histological observation provides the most satisfactory method of comparing the regeneration of the right and left sciatic nerves. It not only permits of a survey of all the factors contributing to the characteristic morphology of the early reparative processes but also allows the application of more objective methods—the measurement of the peripheral advance and density of new axons.

In the early stages (5- and 10-day groups) degenerative changes are more advanced on the left side. This applies not only to the peripheral segments where the fragmentation and absorption of old axons is ahead of the right side but includes a more extensive retrograde degeneration. The difference between the two sides is probably accounted for by the more severe handling of the nerve on the left side.

Axon and myelin fragments are removed slowly and as late as 30 days isolated patches of axon debris still persist in the peripheral segments—solitary survivors of the earlier degenerative phase.

To test the effect of interference with the blood supply on the peripheral advance of the axons we have compared the length of the axis cylinders in the peripheral segments of the right and left sciatic nerves of each animal. The important factor is therefore the mean difference in the axon length of the two sides of the animals of any one group. It has been shown that at no time up to 30 days was this difference of statistical significance.

Text-fig. 1 gives the regression lines representing the increase in length of the axons of the right and left sides from the 10th to the 30th day after operation. The regression coefficients for the right and left sides showing the mean daily rate of advance of the axons are $3{\cdot}52 \pm 0{\cdot}24$ and $3{\cdot}12 \pm 0{\cdot}12$ mm. respectively. The difference is not significant. The growth rate would of course be more correctly represented as a curve but over our small time span the assumption of a constant rate gives a sufficiently satisfactory approximation provided it is remembered, as stated by Seddon, Medawar & Smith (1943) that 'the representation of the rate of regeneration by a straight line must be taken as nothing more than a sample of a curve'. It should be noted that although there is no statistically significant difference in the length of the axons of the two sides at any one stage, or in their growth rates, yet in three out of four groups (10 days is not considered as there are only two animals) there is a tendency to favour the right side.

Gutman *et al.* (1942), employing the pinch reflex, find a growth rate of $4{\cdot}36 \pm 0{\cdot}24$ mm./day for the axons of the crushed peroneal nerve of the rabbit. Even after allowance for shrinkage during fixation our growth rate of 3·52 would probably fall short of this figure and more closely correspond to the growth rate of $3{\cdot}45 \pm 0{\cdot}16$ mm./day (also given by Gutman *et al.* 1942) after cutting and suture, or to Cajal's figure of 3 to 4 mm./day, calculated from histological observations.

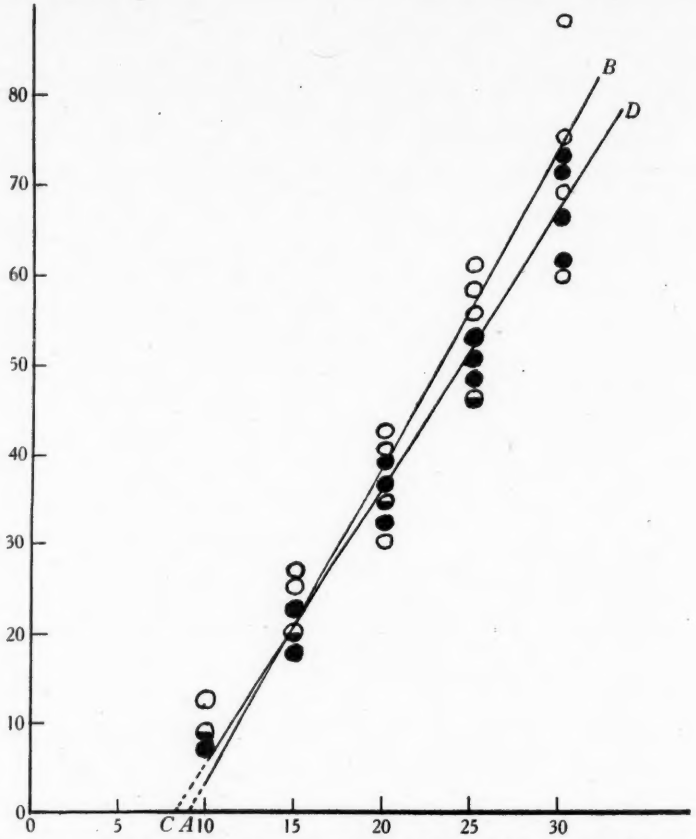
The discrepancy between our figure and that given by Gutman *et al.* (1942) for the crushed nerve probably reflects the difference in the nature of the lesion. The latter crush the nerve with fine smooth-faced watchmakers' forceps which they claim preserves the connective tissue continuity and the general nerve pattern. According to Seddon's (1944) recent work this would be classified as axonotmesis where 'the joining tissue consists of endoneurial tubes and the axons without let or hindrance travel along their old pathways'.

In our experiments we produce a type of lesion which would be included in the neurotmesis of Seddon's (1944) classification. There is almost total disruption of the connective tissue framework and disorientation of the normal nerve pattern. A scar is formed in the crushed area with a 'histological

screen' of blood clot and dense fibrous tissues. In many of our animals, particularly around 15–20 days, there is evidence of frustrated growth such as criss-crossing of fibres, Perroncito spindles, and terminal clubs. These resemble the axon damming and coiling which has been observed by Weiss (1944) in front of a 'throttling bottleneck'.

A more comprehensive and composite picture of the state of regeneration on the two sides can be obtained if in addition the density of the axons is considered. The determination of axon density has not been very widely used in studies of the rate of regeneration.

Greenman (1913), in his work on the regeneration



Text-fig. 1. The plotted points are the lengths of the axons of the right and left nerves of all the animals of the first series. x = days. y = length in mm. ○ = right side. ● = left side. ◒ = a right and left side (where the values happen to coincide). AB = the regression line for the right side. CD = the regression line for the left side.

In the 5-day group there are no new axons in the peripheral segments but by 10 days they are present in considerable numbers and have advanced some distance. The latent period would therefore fall somewhere between 5 and 10 days and agrees approximately with the values for X (when $Y = 0$) obtained by extrapolation (Text-fig. 1).

The axons do not advance on a united front and a comparison of the lengths of the most rapidly growing nerve fibres is but an eclectic measure of the healing progress of right and left sciatic nerves.

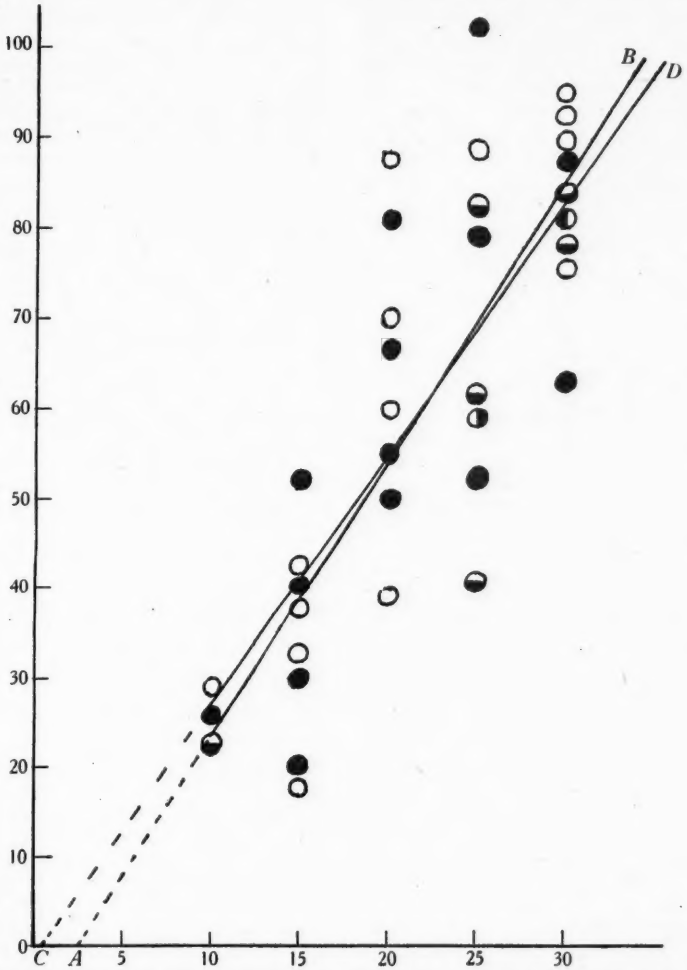
of the peroneal nerve in the rat, gives details of the axon density up to 80 days after operation but as he used osmic acid stained sections his results may be reckoned somewhat selective.

It has been shown that there is no statistically significant difference in the axon density of the right and left nerves at the various stages up to 30 days after operation.

Text-fig. 2 gives the regression lines representing the increase in density of axons for the right and left sides from the 10th to the 30th day after operation.

The regression coefficients for the right and left sides show a mean daily rate of increase of axons of $3{\cdot}09 \pm 0{\cdot}49$ and $2{\cdot}82 \pm 0{\cdot}5$/per mm. respectively. The difference as seen from the figure is not significant.

Extrapolation of the regression lines would deduce latent periods of $2\frac{1}{2}$ days for the right nerve and $\frac{1}{2}$ day for the left nerve. Actual observation shows that new axons do not reach the peripheral segment until some time between 5 and 10 days. This



Text-fig. 2. The plotted points are the axon densities of both sides of all the animals of the first series. x = days. y = no of axons per mm. ○ = right side. ● = left side. ◒ = a right and a left side. ◑ = two right sides. ◐ = two left sides

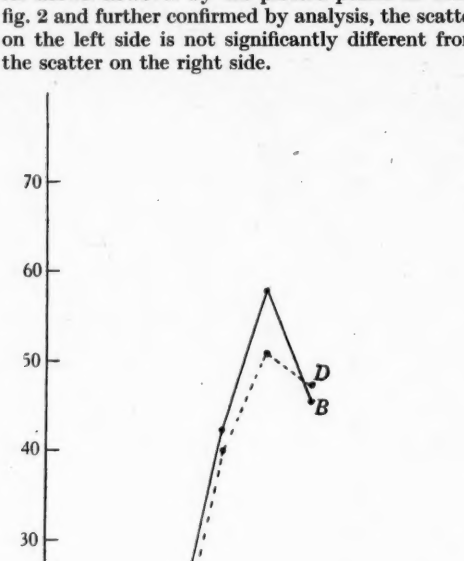
The large standard error compared to that for the rate of increase in the length of the axons can be in part ascribed to our method of determining axon density. It cannot be assumed that there is an even distribution of axons in a cross-section of nerve and the longitudinal sections on which the observations were made are not necessarily equidistant from the central plane.

implies that a logarithmic curve would give a better fit to the observations.

As with the growth rate of the axons the assumption of a constant rate of density increase is probably justifiable over a short interval and 'has some value in elucidating the behaviour of lesions of different types' (Seddon *et al.* 1943).

It might be asserted that the nature of the

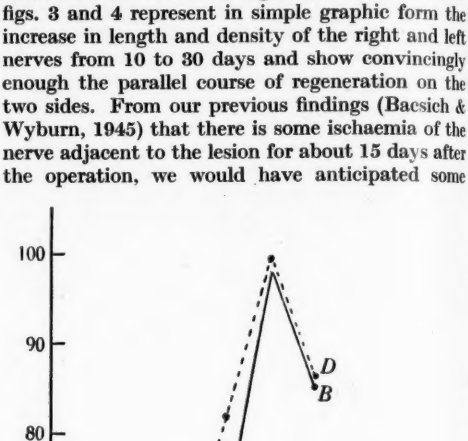
operation on the left sciatic nerve introduces variables which would account for the considerable scatter in the values for the axon density. For instance, during the extensive mobilization there might be some difference in the handling of the nerve—a greater degree of traction or torsion—or some anomaly or irregularity in the blood supply. As shown however by the plotted points in Text-fig. 2 and further confirmed by analysis, the scatter on the left side is not significantly different from the scatter on the right side.



Text-fig. 3. A simple graph showing the lengths of the axons of the right and left nerves of the second series of animals. x = days. y = length in mm. AB = right side. CD = left side.

The figures obtained for the length and density of axons in animal 29, both considerably less than the mean for that group, encouraged us to seek a correlation between them. However, analysis of our whole series of results failed to establish any correlation between length and density except as a function of time.

The results of the second series of experiments reveal that even if the epineural longitudinal anastomoses are destroyed in addition to depriving the nerve of its collateral nutrient vessels, there is no delay in the rate of regeneration. Admittedly the number of experiments (one animal for each period) is small but any adverse effect would be disclosed somewhere throughout the series. Text-figs. 3 and 4 represent in simple graphic form the increase in length and density of the right and left nerves from 10 to 30 days and show convincingly enough the parallel course of regeneration on the two sides. From our previous findings (Bacsich & Wyburn, 1945) that there is some ischaemia of the nerve adjacent to the lesion for about 15 days after the operation, we would have anticipated some



Text-fig. 4. A simple graph showing the number of axons per mm. of the right and left nerves of the second series of animals. x = days. y = no. of axons per mm. AB = right side. CD = left side.

retardation or other evidence of disturbed regeneration. It must however be concluded that the temporary ischaemia produced is neither severe nor prolonged enough to alter materially the normal sequence of events.

It seems desirable to stress again that these results do not imply that peripheral nerves have a privileged autonomy and are independent of their blood supply. Rather do they emphasize the importance of the blood supply, expressed in a flexibility of the vascular arrangement which permits of the maintenance of a normal circulation despite the loss of much of the usual source of supply. When the regional nutrient vessels are

destroyed the epineural longitudinal channels deal efficiently with the emergency. If they in turn are put out of action the secondary (interfascicular) and tertiary (intrafascicular) longitudinal anastomoses are after a short interval equal to the task of preserving the normal blood flow—possibly aided by ingrowing extraneural vessels.

The results of clinically and experimentally produced ischaemia of a nerve segment disclose the functional unit as nerve fibre plus its blood supply and so reveal the necessity for the existing vascular design, able to adapt itself to varying conditions. The majority of the large nerves of the limbs are embedded in muscle masses and it seems very probable that during active limb movements the blood flow through the collateral nutrient vessels is of an intermittent nature. We conceive that a primary purpose of the vascular pattern of peripheral nerve is to promote a continuous blood flow out of the intermittent inflow during period of muscular and consequently nervous activity.

Adams (1943) distinguishes between remote interference with regional sources of blood supply as in our experiments and the occlusion of a nutrient vessel involving all the intrinsic vascular channels, i.e. the longitudinal anastomoses and intraneural ramifications. Such a condition would constitute an effective vascular block and could be brought about clinically by spreading thrombosis or experimentally by a sufficiently high pressure on a segment of nerve.

This is accomplished in the experiments of Rasmussen & Kredel (1944) by the application of miniature blood pressure cuffs directly to the dog's sciatic nerve. They record demyelination of the affected segment. Denny-Brown & Brennan (1944) cite the work of Grundfest (1936) who showed that within the range of clinical possibility the effects of pressure are always due to ischaemia. From their experiments they conclude that the great variation in the rate and extent of impairment of conduction following pressure on a portion of sciatic nerve is caused by 'uneven pressure gradients in the nerve bundles with consequent variation in the degree of ischaemia due to the escape of some small vessels' and further that the greater the pressure the more rapid the onset of paralysis which they state is an 'expression of corresponding relative degrees of ischaemia rather than a direct consequence of pressure on nerve fibres'. Their work corroborates many of the earlier findings of Lewis, Pickering & Rothschild (1931) and Bentley & Schlapp (1943) and is additional testimony to the functional dependence of nerve fibres on their blood supply.

A study of regeneration in an ischaemic nerve segment would furnish an indication of the influence of the blood supply on the recuperative powers of injured peripheral nerve.

Extensive mobilization is a commonplace in the surgical repair of injured peripheral nerve and where end to end suture can thereby be achieved the functional results are often excellent. There may however be some lingering doubts concerning the effect of cutting the numerous collateral nutrient vessels or damaging the surface anastomosis on the speed and extent of ultimate recovery. The vascular pattern of human nerve resembles that of the rabbit and inasmuch as there is justification for inference from animal experiments so is there reason to view dispassionately the necessary vascular interference within the range of normal surgical procedure.

SUMMARY AND CONCLUSIONS

1. The right sciatic nerve of the rabbit was crushed and the left nerve of the same animal was crushed, mobilized and the collateral nutrient arteries cut.

2. In a second series the right nerve was crushed and the left nerve crushed, mobilized, and not only the collateral nutrient arteries but also the epineural longitudinal anastomoses were destroyed.

3. In both experiments the length and density of the regenerated axons of the two sides were compared and at no period up to 30 days after operation was there any statistically significant difference in the regeneration rates as assessed in this way.

4. These results indicate that the regenerating nerves are not affected by the interference with the blood supply and are in keeping with our previous findings (Bacsich & Wyburn, 1945) that there is no significant difference in the vascular pattern after cutting the regional nutrient arteries.

The expenses of this research were met by a grant from the Rankin Medical Research Fund. We wish to thank Prof. D. M. Blair for many helpful suggestions. The statistical treatment of the data was undertaken by Dr R. A. Robb of the Mathematics Department of Glasgow University. We owe a special acknowledgment to Mr George Marshall for his generous technical assistance.

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EXPLANATION OF PLATES

All the illustrations are untouched photomicrographs of sections of the sciatic nerve of the rabbit.

The figures are photographs of 10 μ longitudinal sections at the level of 2 mm. from the centre of the crushed area, stained by a modified protargol method. As the density counts were made from the thicker 10 μ sections, these, rather than the 5 μ sections, were chosen to illustrate the relative densities. ×320.

R.S. = right side. *L.S.* = left side. *S.C.* = simple crush injury. *C.M.* = crushed and mobilized and the regional nutrient vessels cut. *C.M.S.* = crushed, mobilized, the regional nutrient vessels cut and the longitudinal anastomosis destroyed.

PLATE 1

Fig. 1. Animal 1, 5 days after operation. *R.S.*, *S.C.*
Fig. 2. Animal 1, 5 days after operation. *L.S.*, *C.M.*
Fig. 3. Animal 20, 10 days after operation. *R.S.*, *S.C.*
Fig. 4. Animal 20, 10 days after operation. *L.S.*, *C.M.*
Fig. 5. Animal 22, 15 days after operation. *R.S.*, *S.C.*
Fig. 6. Animal 22, 15 days after operation. *L.S.*, *C.M.*
Fig. 7. Animal 23, 20 days after operation. *R.S.*, *S.C.*
Fig. 8. Animal 23, 20 days after operation. *L.S.*, *C.M.*

PLATE 2

Fig. 9. Animal 24, 25 days after operation. *R.S.*, *S.C.*
Fig. 10. Animal 24, 25 days after operation. *L.S.*, *C.M.*
Fig. 11. Animal 25, 30 days after operation. *R.S.*, *S.C.*
Fig. 12. Animal 25, 30 days after operation. *L.S.*, *C.M.*
Fig. 13. Animal 30, 10 days after operation. *L.S.*, *C.M.S.*
Fig. 14. Animal 26, 15 days after operation. *L.S.*, *C.M.S.*
Fig. 15. Animal 27, 20 days after operation. *L.S.*, *C.M.S.*
Fig. 16. Animal 28, 25 days after operation. *L.S.*, *C.M.S.*
Fig. 17. Animal 29, 30 days after operation. *L.S.*, *C.M.S.*

Fig. 1 Fig. 2

Fig. 3 Fig. 4

Fig. 5 Fig. 6

Fig. 7 Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Fig. 16

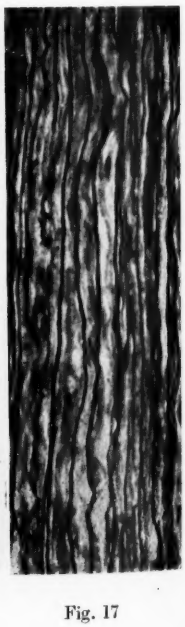


Fig. 17

SOME DATA ON THE EFFECT OF PARATHORMONE ON THE GREY-LETHAL MOUSE

By N. A. BARNICOT, *Department of Zoology, University College, London*

INTRODUCTION

It has been shown that mice homozygous for the recessive gene 'grey-lethal' show greatly reduced bone absorption throughout the skeleton during growth (Grüneberg, 1935, 1936). It is also well known that suitable doses of parathyroid extracts cause bone absorption, though not all species of animal are equally sensitive to this treatment (Jaffe, Bodansky & Blair, 1931; McLean & Bloom, 1937; Bodansky, Blair & Jaffe, 1930). It was deemed of interest, therefore, to study the effect of parathyroid extracts on grey-lethal mice in the hope of throwing some light on the mechanism of the action of the gene and also perhaps on the physiology of parathyroid action. Although not extensive, the following data are presented now because the difficulties of maintaining large stocks of mice during the war have limited the material available and further work must await more favourable circumstances.

MATERIAL AND METHODS

Parathormone (Lilly) was used throughout. This extract contains 0·2 % phenol, but preliminary tests on normal animals showed that this did not affect the weight curves of the animals when repeatedly injected with 0·9 % NaCl. Shortage of material unfortunately made it impossible to carry out more adequate control experiments, using inactivated extracts, and our present controls therefore only cover the effect of injections of large fluid volumes and the disturbance due to repeated manipulations. Injections were in most cases given subcutaneously in the scapular region. Intraperitoneal injections were avoided since with the relatively large volume of fluid leakage may occur on withdrawing the needle. The dosages used are shown in Table 1. Using parathormone (Lilly) a dosage of 70 i.u. per 100 g. body weight involves the injection of 0·17 c.c. of fluid into an animal weighing 5·0 g.; this volume is about as large as can conveniently be injected. The interval between the injections was in most cases about 12 hr., but in some experiments it was as long as 14 hr. or as short as 6 hr. In two cases (Exps. 13 and 14) an interval of 24 hr. was employed. The animals in each series of experiments belonged to the same litter. Since grey-lethals cease to gain weight at about 15 days after birth it is important to start injections as soon as they can be distinguished by their coat colour (at 7–8 days), so that the experiment falls into the period of more active growth. The animals were weighed to the nearest 0·01 g. at the time of each injection. It will be seen from Table 1 that the experiments fall into two

Table 1

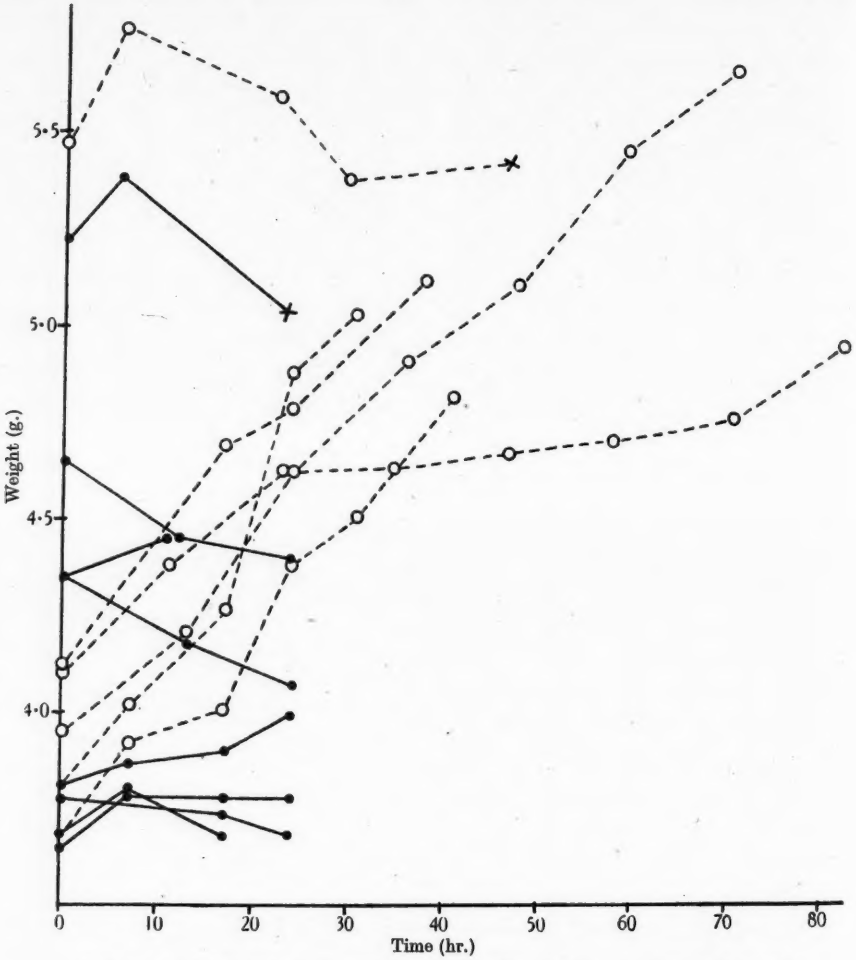
Exp. no.	Dose i.u. per 100 g.	No. of injections	Site of injection	Age at 1st inj. days	Number of animals used: Lethal control	Lethal exp.	Normal control	Normal exp.	Bone destruction: Normal	Lethal
1	70	2	Subcutaneous	9	0	1	0	1	+	+
2	70	2	,,	8	1	2	1	1	+	−
3*	70	2	,,	8	0	1	0	1	+	− ?
4*	70	2	,,	10	0	1	1	1	+	− ?
5	70	3	,,	8	0	1	1	1		− ?
6	70	5	,,	6	1	1	2	3	+	+
7	70	6	,,	10	0	1	1	1	+	+ ?
8*	70	7	,,	9	0	1	0	1	+	+
9*	70	7	,,	7	0	1	1	1	+	+
10*	70	7	,,	8	1	1	1	1	+	+
				Totals	3	11	8	12		
11	20	4	,,	9	1	2	1	2	+	−
12	20	16	,,	11	0	1	1	1	+	=
13	35	2	,,	9	1	1	1	1	=	−
14	1st 3 33 i.u. 2 66 i.u.	5	Intra-peritoneal	9	0	1	2	2	±	−
14	50	1	,,	9	0	0	1	2	+	

* indicates experiments in which material was examined for calcium deposits.

= indicates excess bone deposition.

groups: (*a*) short-term experiments, in which two injections were given, and the normals and lethals sacrificed at the same time, and (*b*) long-term experiments in which the lethals and controls were given as many as seven injections.

Histological technique. The animals were killed by decapitation. Ribs from the middle of the series, the



Text-fig. 1. Weight curve for normal animals. Continuous line, parathormone-injected animals; interrupted line, saline-injected controls.

distal end of the radius, the proximal half of the tibia, the kidney, adrenal, and in some cases the thyroid and parathyroid glands, were fixed in Zenker's fluid with 3% acetic acid. In five of the experiments the heart, lungs, kidneys and adrenals were fixed in formol-alcohol and examined for calcium deposits by the Kossa method. One-half of the skeleton was used for study in cleared preparations stained with alizarin. Zenker material was doubly embedded in celloidin and paraffin, cut serially and stained with Masson's triple stain. Projection drawings of the ribs (Text-figs. 3–5) were made at a magnification of $\times 100$; this is sufficient to allow considerable accuracy of representation, except in the zone of slightly ossified cartilaginous trabeculae.

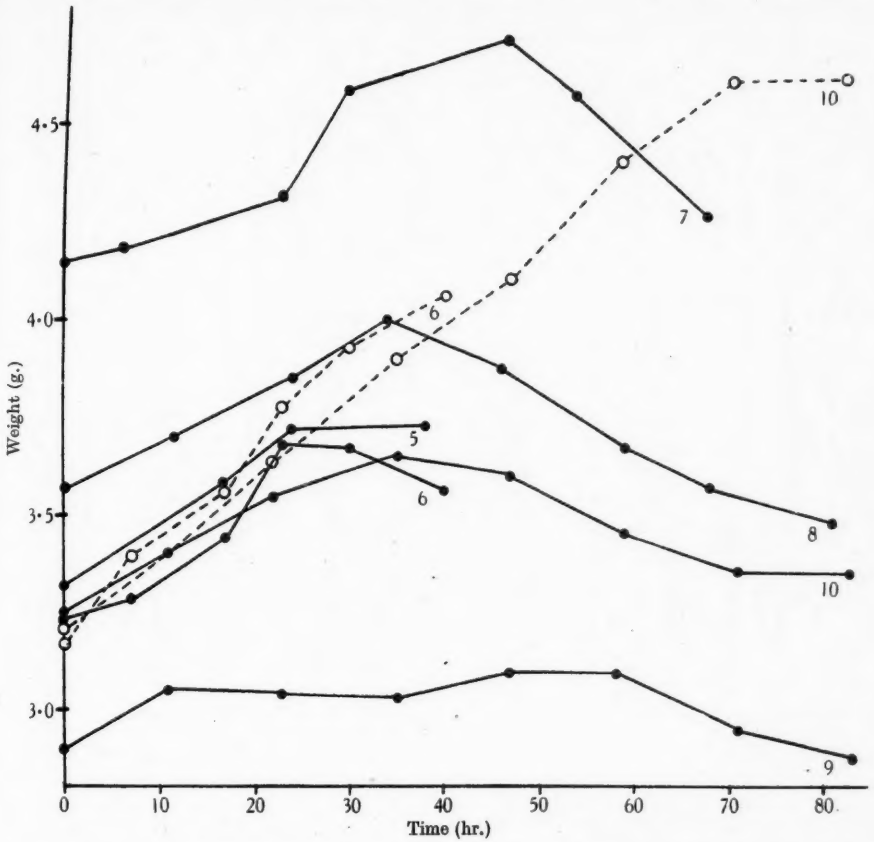
EXPERIMENTAL RESULTS

The weight curves for normal and grey-lethal mice injected with parathormone and also those for the controls, are shown in Text-figs. 1 and 2. It will be

noted that only two lethal control curves are available, but their slope corresponds well with that of the normal controls. Among the normal controls two animals showed a marked decline in weight which was perhaps due to injury inflicted during the injections.

In the case of the parathormone-treated animals it is at once evident that the normal and grey-lethal mice were very differently affected. In the normal series two animals died after one injection, four after two injections and one after three injections; the rest showed marked signs of weakness. Indeed, in the majority of cases injected normals die or become moribund some 12 hr. after their second injection. The body weight usually falls after the first injection, though this decline may be delayed until after the second. Generally no change of behaviour is discovered 12 hr. after the first injection, but after the second injection the animals show various degrees of weakness; e.g. if turned on their backs they right themselves with difficulty or not at all.

The weight curves for lethal animals shown in Text-fig. 2 include only experiments of long duration, using a dosage of 70 i.u. per 100 g. weight. The most notable feature is the number of injections which can be given before the animals show marked signs of weakness. In no case did a lethal die as the result of treatment, and in most cases they were still moving about actively at the end of the experiment. In two cases (Exps. 7, 8) it was noted that the animals' movements were shaky, and in all long experiments there was evident emaciation, but nothing approaching the profound weakness of the injected normals was seen. The weight curves indicate that growth is depressed relatively to that of the controls from the start of the injections, and at

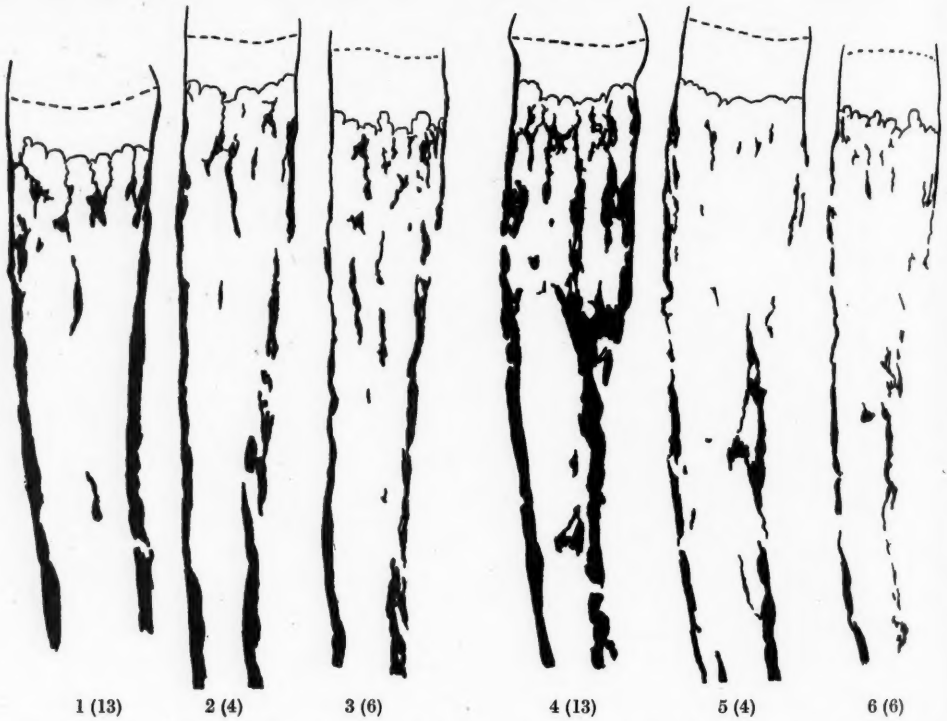


Text-fig. 2. Weight curve for grey-lethal animals. Continuous line, parathormone-injected animals; interrupted line, saline-injected controls. Numbers on curves are experiment numbers (Table 1).

some time between the third and fifth injections the curve begins to fall steadily.

In the case of the experiments in which the dosage of parathormone was 20 i.u. per 100 g. weight, the results so far as normals are concerned differ in no way from those described above. In the case of the lethals the following results were obtained. In Exp. 12, after the seventh injection the weight curve fell and no further injections were given for 48 hr. 20 and 70 i.u., but Exp. 12 suggests that with the lower dosage more injections can be given before the fall in weight occurs.

Two series of injections with a dosage of 35 i.u. per 100 g. at 24 hr. intervals were given to normal and lethal animals. In both cases they were quite active 12 hr. after the second injection, probably because of the longer intervals between injections.



Text-fig. 3. Projection drawings of sections through the ribs of normal animals. The region of the costo-chondral junction is shown by an interrupted line; the numbers in brackets indicate the experimental series from which the experimental material is taken. Nos. 1, 2 and 3 are controls. No. 4 shows excessive bone deposition after two injections of parathormone with a dosage of 35 i.u. at an interval of 24 hr. Nos. 5 and 6 show extensive bone destruction after injections with a dosage of 70 i.u.

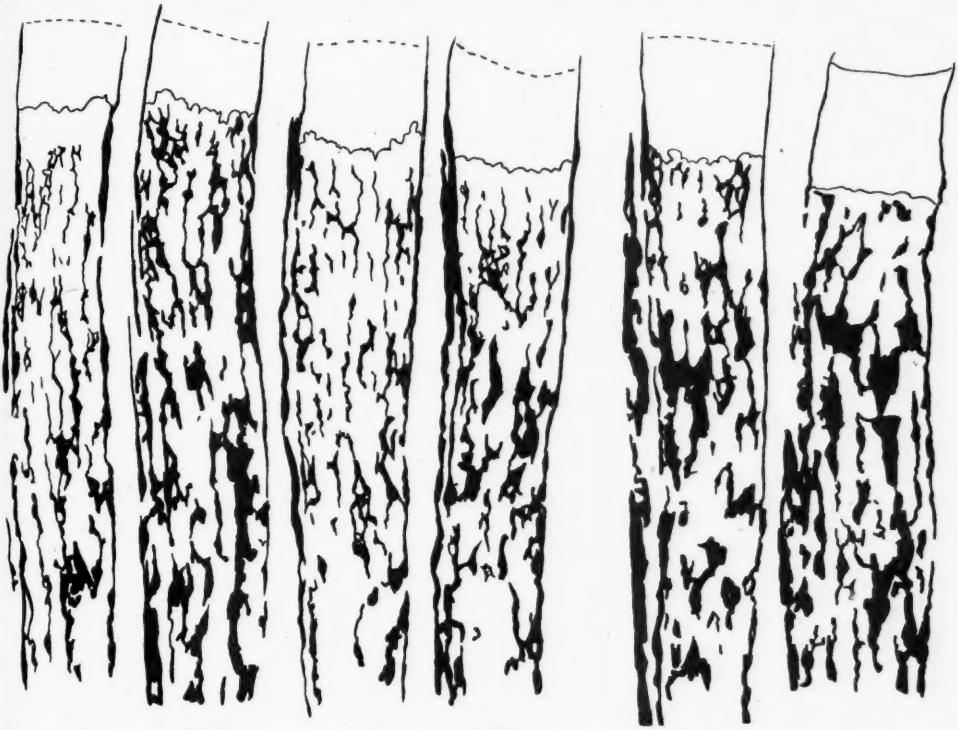
during which the curve rose again. After five more injections the curve again declined. However, the animal was 11 days old at the start of the experiment, and the second half of the experiment may fall within the period of arrested growth which is to be expected in lethal animals. In the series in Exp. 11, only four injections were given and, although the animals remained active, one had begun to fall in weight after the third injection. The data are insufficient to afford conclusive comparison between the groups of lethals injected with dosages of

HISTOLOGICAL RESULTS

The skeleton. The effects of parathormone injections on normal animals will be first described because they do not differ significantly in dosages of 70 or 20 i.u. On the other hand, the results in lethal animals vary with the number of injections and also with dosage. In Text-fig. 3, nos. 1–3 show the normal range of structure found in the costo-chondral region of the ribs of normal control mice. The more extreme effects of parathormone treat-

ment are shown in nos. 5 and 6. In the latter it will be noted that the trabeculae at the junctional zone are cut off from the hypertrophic cartilage and reduced in size and number. Cortical bone is reduced in thickness throughout, leading to the appearance of large gaps, particularly on the deep surface of the ribs. In general, as alizarin preparations show, these gaps occur in those areas of the skeleton where most active bone destruction is already occurring as part of the normal growth pattern. The bone destruction cartilage are often distended with blood, and in more severe cases general haemorrhage may obscure the limits of the vessels. The marrow shows varying degrees of necrosis which evidently starts near the junctional zone (Pl. 1, fig. 2), perhaps due in all cases to mechanical damage. In general, all these effects are well-recognized features of acute hyperparathyroidism.

The effects of parathormone treatment on lethal mice are shown in Text-figs. 4 and 5. The projection



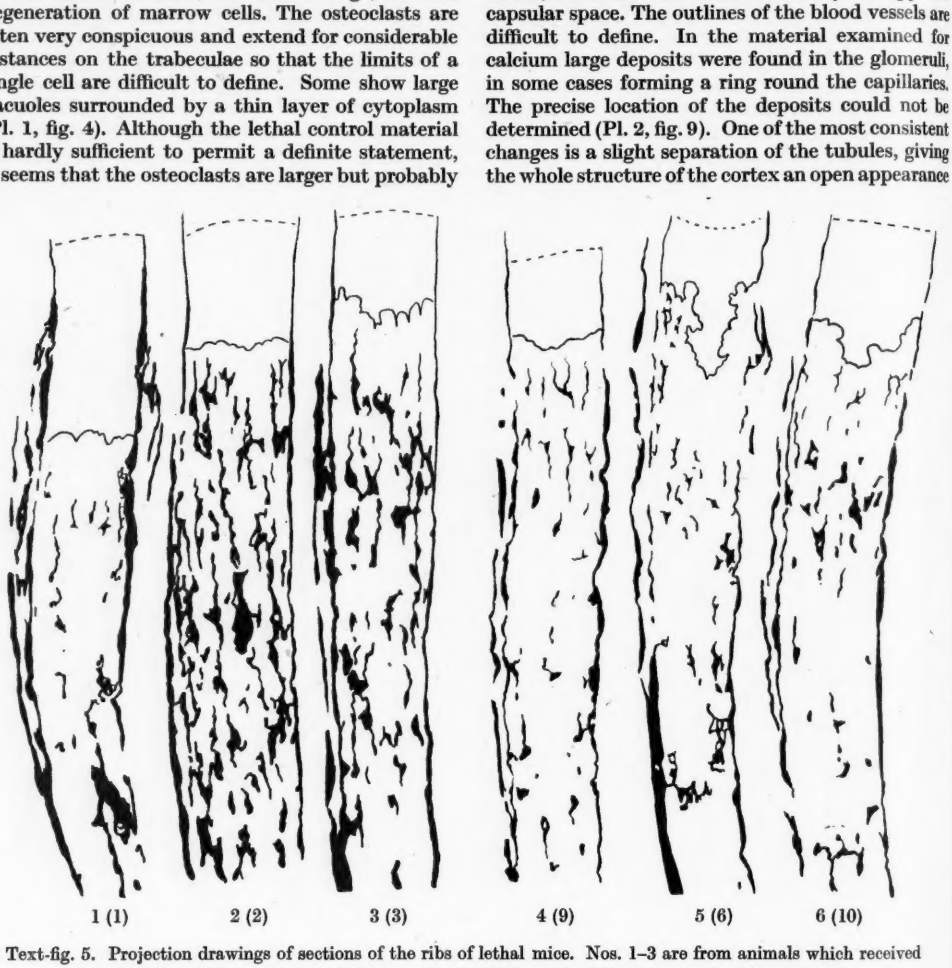
Text-fig. 4. Projection drawings of sections of ribs of lethal mice. Nos. 1–4 are the controls. No. 5 shows an apparent excess of bone deposition after two injections of 35 i.u. at an interval of 24 hr. No. 6 shows excess bone deposition after sixteen injections of 20 i.u.

at a junctional zone in the ribs may be so extensive that fracture occurs, and mechanical injury leads to marrow necrosis in the area. In less severe cases osteoclasts are found applied to the remaining trabeculae. They are usually small and strongly acidophilic, but in the periosteum they are larger and flattened with a pale cytoplasm and vesicular nuclei. The osteoblasts throughout the bone show a striking change from irregularly rectangular basophilic cells to fusiform cells (Pl. 2, figs. 11, 14). The vessels of the marrow and those adjacent to the hypertrophic

drawings in these figures show the great variation in the results, which is partly correlated with the number of injections given. The assessment of bone destruction recorded in Table 1 shows that it is unmistakable in only one of the five experiments in which two injections of 70 i.u. were administered, while in the five experiments in which five to seven injections were given the destruction is well marked in all but one case. Bones from all five experiments with two injections show the following notable features. The osteoblasts everywhere are transformed

into fusiform cells, both in the presence or absence of bone destruction. There is no marked engorgement of the marrow vessels, no haemorrhage, and no degeneration of marrow cells. The osteoclasts are often very conspicuous and extend for considerable distances on the trabeculae so that the limits of a single cell are difficult to define. Some show large vacuoles surrounded by a thin layer of cytoplasm (Pl. 1, fig. 4). Although the lethal control material is hardly sufficient to permit a definite statement, it seems that the osteoclasts are larger but probably not more numerous in the treated animals, and that the occurrence of large vacuoles is a distinctive feature in these animals. The zone of hypertrophic cartilage may be increased in length. Erosion of the cartilage continues longest at the periphery, leaving an axial core of unaffected tissue. The adjacent zone of ossifying cartilaginous trabeculae shows a poor degree of ossification. Osteoclasts are uncommon in this zone and are more numerous where large, well-ossified trabeculae remain.

Soft tissues (*normal animals*). The following abnormal conditions were observed in the kidney of the parathormone-injected normal animals (70 i.u.). The glomeruli show a high proportion of pycnotic nuclei, and some of these cells may occupy the capsular space. The outlines of the blood vessels are difficult to define. In the material examined for calcium large deposits were found in the glomeruli, in some cases forming a ring round the capillaries. The precise location of the deposits could not be determined (Pl. 2, fig. 9). One of the most consistent changes is a slight separation of the tubules, giving the whole structure of the cortex an open appearance under low magnification (Pl. 2, fig. 10). In the controls, the cells of the proximal convolution show acidophilic rods or granules near the base of the cell and a clear area near the lumen of the tubule. In treated animals the cells contain numbers of vacuoles, separated by acidophilic material, which is itself probably formed by the coalescence of large acidophilic inclusions. Granular material is often present in the lumen (Pl. 2, fig. 10). Kossa sections show scattered intertubular deposits throughout the cortex. In two cases (Exps. 3, 8) portions of the proximal convolution were found to be necrotic with



Text-fig. 5. Projection drawings of sections of the ribs of lethal mice. Nos. 1–3 are from animals which received two injections of 70 i.u. and nos. 4–6 from animals which received five to seven injections of 70 i.u.

small and pycnotic nuclei and granular cytoplasm (Pl. 2, fig. 10). The distal convolution may be distended with granular casts but no necrosis was ever observed. Degenerative changes are less evident in the descending limb of Henle's loop than in the proximal convolution. The thin segment may contain granular or hyaline casts and granular material may also occur in the ascending limb. The collecting tubules near the apex of the papilla may be flattened and more darkly staining than normal, and in some cases calcium deposits are present between the tubules in this region. The sites of calcium deposition in the kidney are thus the glomeruli, the intertubular spaces in the regions of the convolutions, and the intertubular spaces near the apex of the papilla.

The adrenal glands show major damage in the injected normal animals, and indeed in four cases were found to be almost entirely degenerated. Massive deposits of calcium are present in the widely dilated sinusoids of both the cortex and the medulla (Pl. 2, figs. 7 and 9). In the control animals the cortical sinusoids are inconspicuous. The cortical cells, which are normally large, clear and finely vacuolated, are compressed in the injected animals by the dilated sinusoids, and their cytoplasm stains darkly, while the nuclei may be pycnotic. In the most severe cases the medullary cells are fragmented and degenerated.

Kossa sections of the heart show numerous calcium deposits between the ventricular muscle fibres and sometimes between the auricular fibres. No deposits are present in the valves or the trunks of the large vessels. Deposits of calcium are also found in the connective tissues of the bronchi deep to the epithelium.

Soft tissues (*lethal animals*). No significant differences were seen in the kidneys of treated (70 i.u. of parathormone) and control lethal animals, and none of the kidneys showed any of the other abnormalities already recorded for treated normals. In one case only were calcium deposits found in parathormone-treated lethal animals and these were situated near the renal papilla. The kidneys of both the controls and parathormone-treated lethals show a feature not seen in normal controls, i.e. large acidophilic granules in the cells of the proximal convolution. Further material, however, is needed to establish the consistency of this feature. In the adrenals neither calcification nor other pathological changes were observed. In both control and treated lethals the glands resemble those of normals except that the zonation of the cortex is less evident and islands of haemopoietic cells are to be found in the cortex and medulla, usually in the region adjacent to the kidney (Pl. 2, fig. 8). This feature, in which the lethals differ from the normals, may be due to the relatively delayed differentiation of the adrenal in the former. In the case of the heart very fine deposits of calcium were found in the atrio-ventricular valves in three cases. This is not a site which shows calcification in treated normals, but since Kossa material of control lethals was not available for study the significance of the observation must remain in doubt. No calcium deposits were found in the myocardium or in the bronchi.

Histological results in lethal animals treated with 20 i.u. parathormone. The two experiments (nos. 12, 13) which come under this heading are not discussed in detail because sufficient comparable data are not available. Certain points which are perhaps significant may, however, be referred to. In Exp. 12 a single lethal animal was given sixteen injections with a pause of 48 hr. after the eleventh. The normal animal given this dosage showed the effects previously described for experiments in which 70 i.u. were used. A rib from the treated lethal animal shows an interesting condition. The trabeculae appear unusually massive (Text-fig. 4, no. 6). The number of osteoclasts is apparently reduced and they are small in size. The osteoblasts had not assumed a fusiform shape. It is known (Selye, 1932; Burrows, 1937) that repeated injections of small doses of parathormone may result in a phase of excessive bone deposition and a state of tolerance to large doses of the extract, so that the bones come to show a condition which at least superficially resembles that found in grey-lethal mice. It is likely that the bone structure described in the lethal animal which received sixteen injections of 20 i.u. is a manifestation of the same phenomenon. In this connexion Exp. 13, in which two injections of 35 i.u. were given with a 24 hr. interval, is of interest. The parathormone-treated normal animal shows excessive bone deposition quite clearly (Text-fig. 3, no. 4), and the osteoblasts are large and rectangular, while the number of osteoclasts as estimated by rough counts seems to be low. Larger numbers of mitoses in the periosteum than are found in normal control bones were also noted. The parathormone-treated lethal animal in this series of experiments showed no definite excess of bone (Text-fig. 4, no. 5), but the osteoblasts were larger than in the bone of the control animals and the osteoclasts were apparently reduced in number. These results suggest that an 'osteoblastic phase' as a response to small doses of parathormone can occur in lethal animals as well as in normal animals of a corresponding age. The possibility that the 'immunity' phenomenon, the mechanism of which is still quite obscure, may have some connexion with the grey-lethal condition, should be borne in mind, but as far as present histological evidence goes there are important differences. In an 'immunized' normal animal the excess of bone trabeculae seems to be the result of new bone encroaching on the marrow cavity, and the osteoclasts are probably reduced in number. In the lethal condition, however, we are dealing with bone which is normally deposited and not subsequently removed.

DISCUSSION

The following major points emerge from these experiments. Grey-lethal mice are not killed or seriously weakened after three times as many injections of 70 i.u. of parathormone as are needed to kill a normal animal. Bone absorption sufficient to be obvious in projection drawings is found in lethal animals after seven, and in one case after two, injections of 70 i.u. Massive bone absorption in lethal mice is not accompanied by severe metastatic calcification in the kidney, adrenal, heart or bronchi. After two injections of 70 i.u., even when bone absorption is not detectable, the osteoblasts of lethal mice show the change to fusiform cells which is characteristic of the bones of injected normal animals.

In injected normal animals the depression of activity, the massive bone destruction and metastatic calcification are conditions which would be expected to be associated with a considerable rise in blood calcium (Bodansky *et al.* 1930; McJunkin, Tweedy & Brenham, 1932). In lethal animals, on the other hand, massive bone destruction can occur without depression of activity or metastatic calcification. Very probably the total amount of bone removed in a lethal animal showing the extreme effects of parathormone treatment is considerably greater than the amount removed in a normal animal of comparable weight, because of the initially greater quantity of trabeculae in the grey-lethal skeleton. However, the assessment of bone absorption by reference to projection drawings is hardly adequate to provide accurate quantitative information. Thus it cannot be said with any certainty after how many injections bone absorption commences in the lethal animal or at what rate it proceeds. Studies on the blood-calcium level, calcium excretion, and the calcium balance are needed for a more exact and conclusive analysis. The results suggest the tentative conclusion that bone absorption does not become marked in the lethal animal until four injections of 70 i.u. of parathormone have been given, and that lower or less frequent doses may never lead to absorption. After seven injections bone absorption is generally massive, and the absence of depression of activity and of calcification may be due to the slower rate of removal of calcium from the skeleton or its more rapid elimination by the kidney, than occurs in normal animals. Thus the blood calcium may never attain a level high enough to produce these effects. It is worth noting that the blood inorganic phosphate in lethal mice has been shown to be high (Watchorn, 1938), and if associated with a low blood calcium this may be a factor in preventing the blood calcium after parathormone treatment from rising to depressive levels. There are, however, few data on the calcium metabolism of mice (Süssman, 1927).

We may suggest the following possibilities: (*a*) the osteoclasts of grey-lethal mice require a higher threshold concentration of parathormone to activate them than those of normal animals; (*b*) the osteoclasts can respond normally in the grey-lethal animal, but the latter in some way eliminates the parathormone more rapidly than a normal animal; (*c*) a combination of both these factors is involved. In regard to the first of these suggestions we know that the rise of blood calcium and the calcium in the urine is proportional to the parathormone dosage in some species (Collip & Clark, 1925; Pugsley, 1932). With small doses the results may be complicated by the 'immunity' phenomenon, but this may be a response which would not normally arise under the action of the animal's own hormone. It is not improbable that a threshold concentration of parathormone has to be reached before the osteoclasts begin to respond, though available evidence does not justify the assumption that this is so. Moreover, previous transplantation experiments (Barnicot, 1941), although open to some doubt in their interpretation, have tended to show that the osteoclasts of grey-lethal animals (or osteoclasts formed from undifferentiated grey-lethal cells) become active when transplanted into normal hosts and when they are therefore presumably exposed to a hormone concentration no greater than that needed to activate osteoclasts of normal animals. Again, the reactions of the osteoblasts of grey-lethal mice to parathormone seem to differ in no way from those of normal animals, suggesting that the hormone concentrations in injected lethals reaches levels which would be adequate to affect these cells as well as the osteoclasts in the normal animal. In the absence of more precise information based on further material, further speculation is hardly profitable at this stage.

There is no evidence that treatment with parathormone in any sense 'cures' the grey-lethal animals of their abnormal condition. It is conceivable that a more precise adjustment of dosage might prolong their life, but the dental condition, for example (which may contribute to their early death), is probably irreversible a week after birth. We also have no clue to the relation between the hair pigment and skeletal abnormalities, and it seems likely that the changes in both these systems have their origin at an early stage of development, involving metabolic disorders as yet unknown.

We have seen that the skeleton of the grey-lethal mouse responds to parathormone if sufficient is given, and we may conclude that the animal's own parathyroid glands (which appear normal in ordinary histological sections) are unable to produce sufficient hormone to affect the skeleton. If we were dealing here with the simple parathyroid deficiency we should not expect that such unusually high doses of parathormone would be necessary to produce bone absorption. In this connexion, cases of pseudo-

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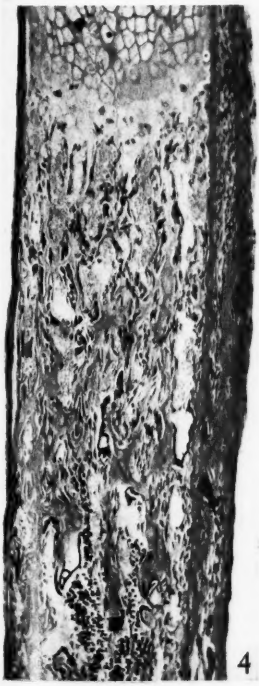
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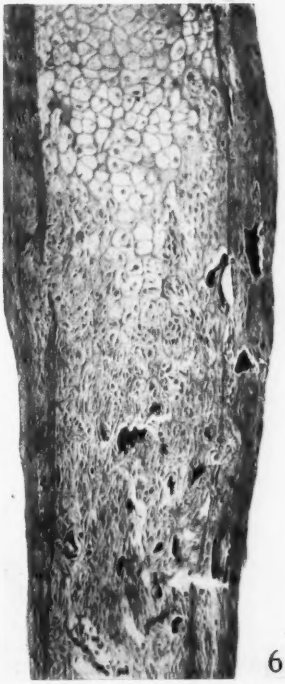
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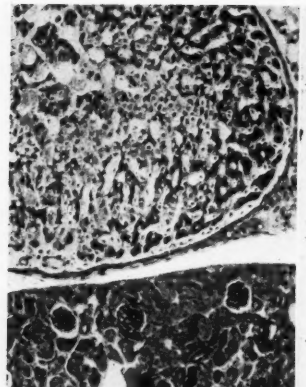
BARNICOT—SOME DATA ON THE EFFECT OF PARATHORMONE ON THE GREY-LETHAL MOUSE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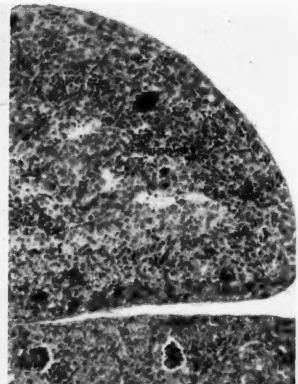
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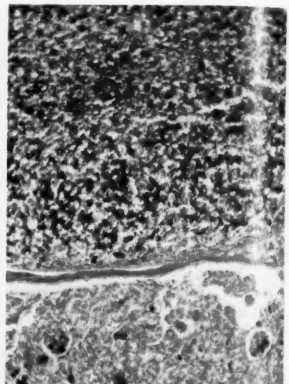
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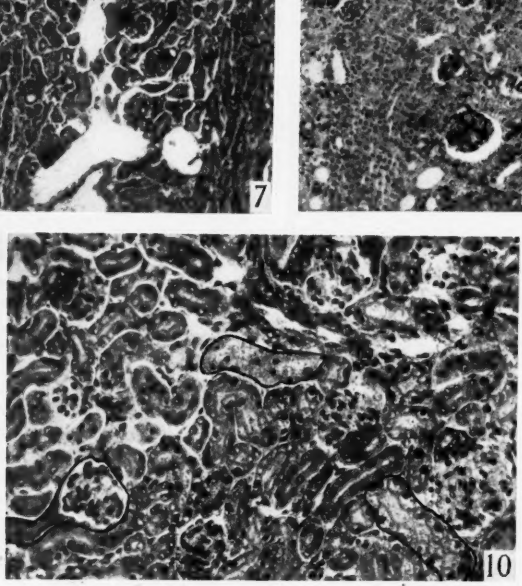
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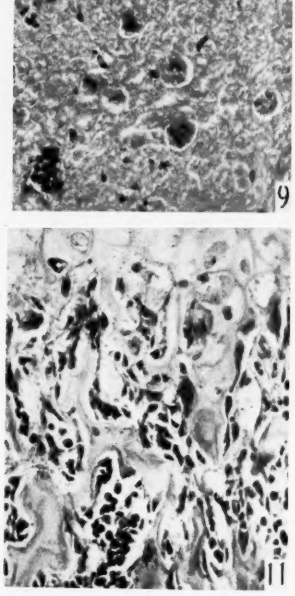
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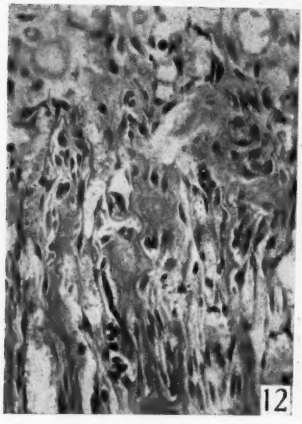
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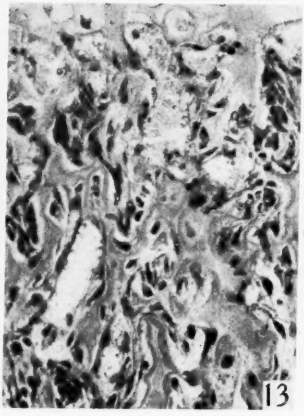
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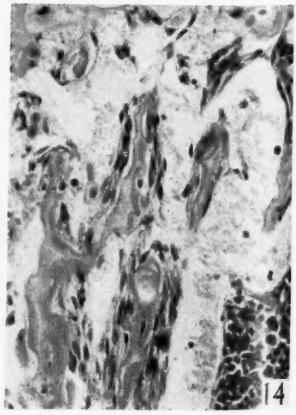
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hypoparathyroidism (Albright, Burnett, Smith & Parson, 1942) with low serum calcium and abnormal tolerance to parathormone may be of interest. In any case experimental parathyroidectomy (though this has been mainly done on much older animals) has never been shown to produce a skeletal condition resembling that of grey-lethal mice.

SUMMARY

1. Grey-lethal mice injected at 12 hr. intervals with 70 i.u. of parathormone tolerate as many as seven injections without depression of activity (though ultimately with a fall in weight), whereas normal animals are killed or severely weakened by two injections.

2. In general, severe bone destruction, often involving removal of nearly all the excess bone which lethal animals show, is found after seven injections but not as a rule after two. Even after two injections osteoblasts in lethal animals show a transformation to fusiform cells which is also a constant feature of parathormone-treated normal animals.

3. Severe bone destruction in the lethal animal is not accompanied by metastatic calcification, whereas in the normal animal the kidney, adrenal, heart and bronchi all show deposits of calcium.

4. Some experiments with lower or less frequent doses are also described. Evidence is presented that low doses lead to an 'osteoblastic phase' with excessive bone deposition in both lethal and normal animals.

5. The interpretation of the results is discussed.

This work was started in the Department of Zoology and finished in the Department of Anatomy, University College, London. I have to thank Dr Grüneberg for his interest and also Prof. J. B. S. Haldane, Department of Biometry, University College, London, Prof. Spaul, Department of Zoology, University of Leeds, and Dr E. C. Amoroso, Department of Embryology, Royal Veterinary College, London, who gave me various facilities for continuing the work in the intervening period, and Mr F. J. Pittock for the photographs.

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EXPLANATION OF PLATES

Plate 1

Sections of ribs near the costo-chondral junction. Masson triple stain. The osteoclasts have been blacked in.

Fig. 1. Normal control.
Fig. 2. Normal rib after treatment with 70 i.u. parathormone. The trabeculae are cut off from the cartilage; the cortex is thin and irregular; the marrow is disorganized and necrotic.
Fig. 3. Grey-lethal control. Well-ossified trabeculae are seen in the marrow cavity; large osteoblasts lie near the cartilage.
Fig. 4. Grey-lethal rib after two injections of 70 i.u. parathormone. There is no obvious bone destruction and the osteoblasts are fusiform; the osteoclasts are large and some contain large vacuoles.
Fig. 5. Grey-lethal rib after seven injections of 70 i.u. parathormone. A few well-ossified trabeculae are seen in the proximal region of the marrow cavity and the cortex is thin and irregular; the osteoblasts are fusiform; the distal part of the marrow contains fine cartilage trabeculae and there is an axial projection of the hypertrophic cartilage.
Fig. 6. Grey-lethal rib after 70 i.u. parathormone. Very few islands of well-ossified bone remain; the distal part of the marrow contains a relatively avascular tissue of fusiform cells lying between fine cartilage trabeculae. There is a well-marked axial projection of hypertrophic cartilage and some very large osteoclasts.

Plate 2

Fig. 7. The adrenal gland and adjacent renal cortex of a parathormone-treated normal animal. Masson triple stain. Note the separation of the renal tubules and the dilated sinusoids and shrunken cells of the adrenal cortex.
Fig. 8. The adrenal gland and renal cortex of a grey-lethal animal after seven injections of 70 i.u. parathormone. There is no evidence of damage. Note the islands of haemopoietic tissue in the adrenal.
Fig. 9. Kossa-stained section of the adrenal gland and renal cortex of a normal animal treated with 70 i.u. parathormone. Massive calcium deposits are present in the adrenal, and glomerular and intertubular deposits in the renal cortex.
Fig. 10. Renal cortex of a parathormone-treated normal animal. Masson triple stain. Note separation of the tubules, pycnotic cells in the glomeruli, a portion of proximal convoluted tubule with vacuolated and granular material in the lumen (outlined bottom right) and a portion of necrotic proximal convolution (outlined near centre of field).
Fig. 11. Normal control bone. The section is taken through the trabeculae near the hypertrophic cartilage and shows the form of the osteoblasts.
Fig. 12. Parathormone-injected grey-lethal bone, showing fusiform shape of osteoblasts.
Fig. 13. Grey-lethal control bone, showing the form of osteoblasts.
Fig. 14. Parathormone-treated normal bone. Note the widely dilated sinusoids and fusiform osteoblasts.

A SIMPLE METHOD OF VASCULAR INJECTION AND ITS APPLICATION IN PREPARING BRAIN SECTIONS

By O. E. NICHTERLEIN, *Department of Anatomy and Histology, University of Adelaide*

In the study of the vascular pattern of different organs, many of the injection techniques employed suffer from the disadvantage that they disturb radically the condition of the circulation in the vessels to be observed. As Pfeiffer (1930) and others have pointed out, it is desirable that certain conditions should be fulfilled, particularly that the hydrodynamics of normal blood flow should be imitated as closely as possible. Hence the viscosity of the injection fluid should approximate that of blood, and the normal range of pressure within the vessels should not be exceeded. It will obviously be an advantage if the injection can be done in a living animal.

A method of injection has been devised which approximates to these requirements, viz. the injection of a silver proteinate solution into the heart of the living animal. B.D.H. Argenti Proteinas mite (19–25 % Ag) was used. A 25 % solution was found to be of the same viscosity as sheep's blood, and was the strength generally adopted, although weaker solutions also gave satisfactory results. The method was employed only for cerebral injections, but it is applicable to any vascular region.

TECHNIQUE

Cerebral injection. The following steps were found most convenient in the mammals injected, viz. rat, rabbit, opossum, cat, dog, and sheep:

(1) Under anaesthesia cut through the upper costal cartilages to open the thorax widely, keeping far enough to the left of the midline to avoid the internal mammary vessels.

(2) Pull the apex of the left lung forwards or laterally to display the aortic arch. Clamp the aorta distal to the arch.

(3) Clamp the inferior vena cava medial to the base of the right lung.

The last two steps cut off the circulation of the lower portion of the body. Doing so saves injection material and ensures that the whole force of the heart will be employed in getting the injection through the remainder of the body. If it is thought undesirable to disturb the circulation in this way these steps can be omitted.

(4) Open the pericardium and secure the tip of the right auricle in a pair of ratchet forceps.

(5) Having the injection fluid ready in a hypodermic syringe of suitable size, with no air in the needle, insert the needle into the left ventricle.

(6) Cut the tip of the right auricle and begin to inject the solution. Inject steadily, keeping the pressure low enough not to distend the heart. The heart itself will pump the injection fluid through the portion of the circulation which has been left intact. Blood and excess injection fluid will flow out through the right auricle. If in doubt whether the injection is running properly, observe the colour of the sclera of the eye or of the mucous membrane of the mouth.

Quantities injected varied from 1 c.c. for the rat to 5–10 c.c. for the rabbit and opossum and 20 c.c. for a young lamb. These quantities, which are roughly equivalent to 3 c.c. per kg. body weight, were found satisfactory, but they can be varied within fairly wide limits.

(7) After injection place the animal so that the head is in a dependent position for an hour or so. A ligature may be put round the neck to help to prevent loss of fluid.

After this the brain is removed and fixed in formalin. Care must be taken in handling the brain as pressure on the brain surface will tend to drive the fluid out of the vessels.

Modifications necessary for the injection of other organs or parts of the body are easily devised.

It is obvious that the normal conditions of hydrodynamics are not completely preserved in the use of a technique involving such extensive circulatory disturbance as cutting off a large portion of the circulation and opening the right auricle. It is doubtful whether any injection technique can be perfect in this respect. At the same time this method offers a considerable advantage in this regard over such as require the use of higher pressures or are performed on the dead animal.

Embedding. For embedding, a modification of Bennett's (1940) rapid amyl acetate method was used with success. The only pyroxylin available was the commercial product, and as its viscosity in solution was found to vary considerably from sample to sample, it was impossible to work in percentages in making up thick and thin solutions.

Chloroform was found unsuitable for hardening the blocks as it had two defects. One was a tendency for cheesy holes to form in the chloroform-hardened blocks. The other, a more annoying one, was due to the fact that the chloroform, as it displaces the amyl acetate, raises the specific gravity of the pyroxylin solution before it hardens it; consequently nerve tissue floats to the top of the solution and its orientation within the block

alters. Both these defects were remedied by using benzol as a hardening agent. This also cleared the block.

By allowing sufficient pyroxylin above the specimen, the finished block could be clamped into the block holder of a sliding microtome without mounting on a carrier. Sections at 300 μ were cut without difficulty. The block was irrigated with xylol while being cut. As the sections were already cleared, they were ready for immediate mounting.

They were mounted between sheets of thin glass salvaged from old lantern slides. A number of sections were mounted on each slide in fairly thin Canada balsam, kept warm to decrease its viscosity. The difficulty of preventing thick sections from drying out was obviated by putting a 'gasket' of thin blotting paper round the edge of the slide. The numbers of the sections and other relevant details were written on the 'gasket' in India ink.

After mounting and covering, the slides were left weighted on the hot-plate overnight. Later the edges were scraped and covered with bituminous cement.

This technique gives a complete and uniform capillary injection, brown in colour, and turning darker on exposure to heat or sunlight. In the larger vessels, and possibly in the smaller ones, the silver seems to stick to the endothelium rather than to fill the lumen.

The sections photograph readily, and it is possible to make prints for low power reproduction by using the section as a negative in a photographic enlarger.

The technique is reliable and very simple. India ink or other injection fluid can be used with the same technique, though India ink seemed to give a less perfect injection than silver. The procedure described for cutting and mounting does away with much handling of the material.

It might be possible to use the silver injection for measuring the relative vascular densities of tissues by doing silver estimations on equal weights of injected unfixed tissue.

SUMMARY

1. A simple and effective method of vascular injection has been described using a silver proteinate solution as the injection fluid, and injecting directly into the heart of the living animal.

2. A modification of Bennett's rapid amyl acetate method of embedding has been found effective.

3. A procedure for cutting and mounting is described which is suitable for thick sections.

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IN MEMORIAM

DUNCAN MacCALLUM BLAIR, M.B., ChB., D.Sc., F.R.F.P.S.G., F.R.S.E.,
Regius Professor of Anatomy, University of Glasgow

Professor Blair died suddenly on 10 November 1944, at the early age of 48. By his death the Anatomical Society has lost a member who made significant contributions to the science of anatomy and who will live in the memory not only through his academic work but also for that genuine and generous friendliness which gave him warm welcome from all men at all times. He was the son of Dr Alexander Blair of Ashington, Northumberland, and Crinan, Argyll. He entered Glasgow University as a medical student in 1913, and during his course of studies he was awarded many prizes and distinctions, including the Asher-Asher Gold Medal in Oto-rhino-laryngology. His studies were interrupted by the 1914–18 war; he served as a Surgeon Sub-Lieutenant in the Royal Naval Volunteer Reserve from 1915 to 1917, and in 1919 he graduated M.B., Ch.B. with Honours. In the latter year he was appointed Assistant to Professor T. H. Bryce, and in 1922 he was promoted to the Senior Lectureship in the Anatomy Department of Glasgow University. Blair's first ambitions were directed towards surgery, and he spent much time in the surgical units of the Glasgow Western Infirmary, where he had previously acted as House Surgeon to Sir Kennedy Dalziel, but Professor Bryce persuaded him to devote his whole time to anatomy. At this time Blair and his colleagues carried out the dissections on which many of the diagrams and much of the description in the eleventh edition of the Myology Section of Quain's *Elements of Anatomy* are based. As Senior Lecturer, Blair was primarily responsible for the introduction of histology (including neuro-histology) into the anatomy course, and the Glasgow School thus became one of the pioneers in the restoration of the teaching of this subject to the anatomical department in this country.

In 1927, when 31 years of age, he succeeded Professor E. Barclay Smith in the Chair of Anatomy in the University of London at King's College. Here he organized the anatomy course much on the lines pursued at Glasgow, and he laboured day and night in planning the construction and equipment of the new Hambleden Department of Anatomy. It is a tribute to him that a number of new anatomical departments built subsequently in this country were based to a considerable extent on the model of Blair's department at King's. While in London Blair also acted as John Hunter Lecturer in Applied Anatomy at St George's Hospital Medical School. In 1928 he married Leonie Elspet, daughter of James Stewart of Aberdeen, and there are two daughters. He succeeded Professor Bryce as the Regius Professor of Anatomy in Glasgow, and he quickly applied the lessons he had learned at King's College in extending the equipment at Glasgow.

Blair acted as examiner in anatomy in the Universities of Aberdeen, Glasgow, London, St Andrews and Sheffield, as well as for the Royal College of Physicians of London. His research interests were wide, and, among other things, he published papers on the arrangement of the fibres of the central tendon of the diaphragm, the nerve supply of arteries, the types of nerve cells in dorsal root ganglia, macroscopic staining reactions of the brain, the preparation of dry specimens by paraffin-naphthalene impregnation, the use of infra-red photomicrography in neuro-histology, the causation of vascular symptoms in cases of cervical rib, and the conducting system of the mammalian heart. He was the author of the section on Arthrology in the recent editions of Cunningham's *Textbook of Anatomy*. In 1923 he shared the Symington Prize for anatomical research, and he was later elected a Fellow of the Royal Society of Edinburgh and a Fellow of the Royal Faculty of Physicians and Surgeons of Glasgow. For his published research he was awarded the Doctorate of Science of the University of London in 1935.

As a teacher and administrator, Blair had two outstanding characteristics, sound common sense and a real sense of proportion. He excelled in the exposition of his subject, and his impressive, clear, fluent and concise delivery made his lectures a feature of the medical course and a lasting inspiration to his students. His breadth of vision and his wise counsel caused him to be eagerly sought by those who had to deal with difficult administrative problems. He acted (in rotation) as Dean of the Faculty of Medicine at King's College, London, and gave valuable services to the Anatomical Society as a Member of Council, of the Committee of Management, and of the Symington Bequest Committee. In view of present-day discussions concerning future methods of teaching medical students (e.g. especially the suggestion in the *Report of Inter-departmental Committee on Medical Schools*, p. 125, para. 51), it is interesting to note that a decade ago Blair interested himself in those problems and wrote for the *Medical Press and Circular* (1934, **188**, 225) on 'The function of anatomy in the medical curriculum'. In this article he suggested that anatomy and physiology

DUNCAN MacCALLUM BLAIR

1896–1944

were tending to lose their more or less equivalent status as complementary subjects in the curriculum, physiology rapidly becoming a specialist outgrowth while anatomy retained its position as a general subject in the main stem of the medical course. Blair envisaged a day when neither anatomy nor physiology would occupy a separate department, but, instead, a combined department with specialists in embryology, topography, neurology, biochemistry, metabolism, etc., would come into existence.

With the advent of the present war Blair became involved in many medical duties connected with Civil Defence. As a boy he spent much of his time on the shores of a Scottish loch and there acquired a love of the sea and ships which not even the rigours of service on a minesweeper in the 1914–18 war could lessen. When invited in 1942 to undertake the organization of the Glasgow University Naval Division he accordingly applied all his abilities to this task, and after two years of strenuous work had under his command one of the most efficient and successful University Training Units.

It was characteristic of his interpretation of his duties as head of the department that while in Glasgow he put aside many of his own personal scientific pursuits that he might the more freely help and advise the work of others. Keen to encourage and foster the spirit of inquiry and research in his staff, he devoted much time to inspire, direct, and guide their efforts.

There could be no more fitting tribute to Blair than that of the Principal of Glasgow University, Sir Hector Hetherington: 'In every University and in every generation there arise one or two men to whom it is given by some especial energy and grace to kindle and quicken the life around them. Of this good company was Duncan Blair. A master of his subject, a teacher of rare enthusiasm and power, a lover of the sea and open spaces, by native genius a leader of men, strong in conviction but generous as the day and full of wholesome laughter, he drew inevitably the affection and loyalty of students and colleagues alike. . . . I have not known a man happier in home, in work, in public duty or more constant in his reliance on that Christian faith which was to him the source of his serenity and strength.'

To his wife and daughters we can but extend our heartfelt sympathy.

F.D.
G.M.W.

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